HHS Public Access

Author manuscript

Mol Microbiol. Author manuscript; available in PMC 2019 February 01.

Published in final edited form as:

Mol Microbiol. 2018 February ; 107(3): 277–297. doi:10.1111/mmi.13887.

Overview of carbon and nitrogen catabolite metabolism in the virulence of human pathogenic fungi

Laure Nicolas Annick Ries[1], **Sarah Beattie**[2], **Robert A. Cramer**[2], and **Gustavo H. Goldman**[3]

[1]Faculdade de Medicina de Ribeirão Preto, Universidade de São Paulo, Av. Bandeirantes, 3900, CEP 14049-900, Ribeirão Preto, São Paulo, Brazil

[2]Geisel School of Medicine at Dartmouth, Department of Microbiology & Immunology, 74 College Street Remsen 213, Hanover, NH 03755, USA

[3]Faculdade de Ciências Farmacêuticas de Ribeirão Preto, Universidade de São Paulo, Avenida do Café s/n°, CEP 14040903, Ribeirão Preto, São Paulo, Brazil

Summary

It is estimated that fungal infections, caused most commonly by *Candida albicans, Aspergillus fumigatus* and *Cryptococcus neoformans*, result in more deaths annually than malaria or tuberculosis. It has long been hypothesized the fungal metabolism plays a critical role in virulence though specific nutrient sources utilized by human pathogenic fungi *in vivo* has remained enigmatic. However, the metabolic utilisation of preferred carbon and nitrogen sources, encountered in a host niche-dependent manner, is known as carbon catabolite and nitrogen catabolite repression (CCR, NCR), and has been shown to be important for virulence. Several sensory and uptake systems exist, including carbon and nitrogen source-specific sensors and transporters, that allow scavenging of preferred nutrient sources. Subsequent metabolic utilisation is governed by transcription factors, whose functions and essentiality differ between fungal species. Furthermore, additional factors exist that contribute to the implementation of CCR and NCR. The role of the CCR and NCR-related factors in virulence varies greatly between fungal species and a substantial gap in knowledge exists regarding specific pathways. Further elucidation of carbon and nitrogen metabolism mechanisms is therefore required in a fungal species- and animal model-specific manner in order to screen for targets that are potential candidates for anti-fungal drug development.

Introduction

It is estimated that infections caused by the fungal pathogens *Candida albicans, Aspergillus fumigatus* and *Cryptococcus neoformans*, kill more people annually than malaria and

Corresponding authors: Drs. Gustavo H. Goldman and Laure Nicolas Annick Ries, ggoldman@usp.br and rieslaure13@gmail.com, Tel: +55(16)3315428.

Author contributions

GHG, RAC, SRB and LNAR contributed to the conception and design as well as the analysis and interpretation of the data of this manuscript. LNAR and SRB wrote the manuscript.

Conflict of interest

The authors declare that no conflict of interest exists.

tuberculosis (Denning and Bromley, 2015; Meyer *et al.*, 2016). These fungi cause a spectrum of diseases, ranging from superficial to systemic and invasive, depending on the underlying disturbance in host defence responses and physiology (Mayer *et al.*, 2013; Abad *et al.*, 2010; Lee *et al.*, 2013).

Pathogenicity, the ability to cause disease, is a multifactorial trait which encompasses a variety of survival and fitness-enhancing factors and pathways that determine the virulence of a pathogen in a given host. Among these, nutrient acquisition and subsequent metabolic processes are a critical virulence determinant as they are essential for promoting fungal fitness, survival, and virulence within the host. Essential nutrients include minerals such as iron and zinc which are required in small amounts, while carbon and nitrogen, the main energy sources for sustaining biosynthetic processes, must be obtained in large quantities from the environment (Ramachandra *et al.*, 2014). Fungi have preference for certain carbon and nitrogen sources that are rapidly metabolised and therefore provide quick energy for growth and niche colonisation (Ruijter and Visser, 1997; Schure *et al.*, 2000). In the presence of these favoured energy sources, the utilisation of alternative, less preferred carbon and nitrogen sources may be repressed, a process known as carbon and nitrogen catabolite repression (CCR, NCR). This review presents an overview of the current literature on CCR and NCR, including sensing and uptake of preferred carbon and nitrogen sources, whilst emphasising the importance of these regulatory mechanisms for the pathogenicity and virulence of *C. albicans, A. fumigatus* and *C. neoformans*.

In vivo carbon and nitrogen source availability

In the context of infection, the main carbon sources available to pathogenic fungi are glucose, lactate and acetate, whose availability largely depends on the host niche. In addition, potential nitrogen sources can also serve as carbon sources and are available throughout the human host mainly in the form of proteins. The primary site of infection for *A. fumigatus* and *C. neoformans* is the respiratory tract and occurs through inhalation of fungal spores. The airway surface liquid (ASL) of the lung and nasal secretions typically contain less than 0.5 mM glucose in healthy individuals, about 10-fold less than serum (Philips *et al.*, 2003, Baker *et al.*, 2007). In addition, lactate and amino acids have been identified in the ASL of mice and concentrations of these metabolites increase with inflammation (Grahl *et al.*, 2011, Hu *et al.*, 2008). Furthermore, ammonium, a preferred nitrogen source that enters the human body either as dietary free amino acids or is produced locally by bacterial microbiomes of the gastrointestinal (GI) tract (Richardson *et al.*, 2013) and lungs, can also be found in the lung airways. Underlying respiratory diseases, such as cystic fibrosis, significantly increase local ammonium concentrations (Gaston *et al.*, 2002). The lung mucous layer is composed of different glycoproteins such as mucin, which likely present good carbon/nitrogen sources for fungi. Fungal-induced damage to the mucosal barriers allows access to the underlying connective tissue which mainly consists of the structural proteins collagen and elastin (Farnell *et al.*, 2012; Kronstad *et al.*, 2012). A similar situation can be envisaged for *C. albicans* when switching from a gastrointestinal (GI) and vaginal commensal organism to a pathogenic organism, inducing penetration of the gut and vaginal mucosal membranes and gaining access to the underlying tissue.

Further tissue-induced damage would subsequently result in all three fungi reaching the bloodstream, which typically contains glucose and lactate levels of 4–5.5 mM and 0.5–2mM, respectively, depending on diet, and medical conditions such as diabetes and cystic fibrosis, which increase serum glucose levels (Baker *et al.*, 2006, Ament *et al.*, 1997). Furthermore, interorgan transport ensures the distribution of amino acids throughout the body which are required for protein synthesis and specific metabolic functions (Brosnan, 2003). The concentration of plasma amino acids is around 2.5 mM with glutamine being the most abundant amino acid in the blood (Brosnan, 2003) and in the intracellular amino acid pool of most tissues (Stumvoll *et al.*, 1999). Dissemination via the bloodstream enables organ colonisation; in the case of *C. neoformans*, access to the cerebrospinal fluid (CSF), crossing of the blood brain barrier (BBB) and invasion of the brain. The organic acids lactate and acetate are produced by bacteria of the human gut or in a tissue-dependent manner, especially during inflammation where infiltrating immune cells primarily produce lactate for ATP generation from glucose (Jiménez-López *et al.*, 2013; Schug *et al.*, 2016) (Borregaard & Herlin, 1982). In the CSF, glucose levels are typically about 60–70% of serum glucose levels (Seehusen *et al.*, 2003). Additionally, the levels of carbon and nitrogen sources *in vivo* change with corticosteroid treatment, a risk factor for many fungal infections, and upon inoculation with fungal conidia (Beattie *et al.*, 2017). An important area for further research is how medical treatments that pre-dispose individuals to fungal infections alter the nutrient environment of specific organ systems. Thus, in addition to being able to utilize available carbon sources, pathogens must also be able to adapt to a rapidly changing nutrient environment within the host. Therefore, when considering human pathogenic fungi, we must examine metabolism of both glucose and alternative, non-preferred carbon and nitrogen sources. Figures 1 and 2 give an overview of known factors involved in carbon and nitrogen metabolic utilization.

Carbon catabolite metabolism in human pathogenic fungi

Glucose sensing and uptake

Given the importance of glucose in fungal metabolism, fungi have evolved sensitive systems for the sensing and uptake of glucose. In the well-studied model organism, *S. cerevisiae*, there are two major types of glucose sensing mechanisms: the G-Protein Coupled Receptor (GPCR) system, with the receptor Gpr1p and G-protein Gpa2p, and the hexose transporter gene family members Snf3p and Rgt2p [reviewed in (Peeters and Thevelein, 2014)]. Here we use this well-studied system as a basis for discussion but note that unlike the human pathogenic fungi discussed herein, *S. cerevisiae* is a Crabtree positive organism, i.e., it produces ethanol in aerobic conditions and high external glucose concentrations rather than accumulating biomass via the tricarboxylic acid (TCA) cycle. Thus, additional opportunities exist to discover glucose sensing and uptake mechanisms in the most commonly encountered human pathogenic fungi. In *C. albicans*, Gpr1p/Gpa2p regulate hyphal morphogenesis through activation of the cAMP-PKA (protein kinase A) pathway (Miwa *et al.*, 2004, Maidan *et al.*, 2005a). Whether this receptor is directly sensing glucose is unclear, however evidence suggests that this receptor also responds to methionine to stimulate hyphal formation in response to carbon source (Maidan *et al.*, 2005b). The role of the Gpr1p sensing system in virulence was assessed in two studies, where Maidan and colleagues

(2005a) reported reduced hyphal formation, tissue invasion and virulence of a gpr1−/− strain when injected intravenously in an inbred mouse model of systemic candidiasis, whereas Miwa *et al.* (2004) observed wild type virulence of intravenously-injected hetero- and homozygous mutants of *GPR1* and *GPA2* using an outbred mouse strain in a systemic model of candidiasis. Additionally, each study used a different cell density (optical density) inoculum, thus this signalling pathway may be important for virulence in a host-specific or dose-dependent context. In *C. neoformans*, Gpa2 functions upstream of cAMP-PKA signalling, and is required for cAMP signalling in response to glucose, however, the upstream sensor associated with this G protein remains unknown (Xue *et al.*, 2006) and deletion of *GPA2* did not affect virulence in an inhalation model of murine infection using both immune competent and immune deficient (severe combined immunodeficiency, SCID) mice (Li *et al.*, 2007).

The genome of *A. fumigatus* encodes at least 15 GPCRs, of which only GprC, GprD and GprK, have been characterised (Grice *et al.*, 2013, Jung *et al.*, 2016). GprC and GprD show amino-acid sequence similarity to ScGpr1p, and deletion of either of these receptors results in a severe, temperature-dependent growth defect independent of carbon source (Gehrke *et al.*, 2010, Han *et al.*, 2004). Although no carbon-dependent growth defect of GprD was observed, metabolomic studies demonstrated that loss of the homologue receptor in *A. nidulans* altered glucose and central carbon metabolism (de Souza *et al.*, 2013). Deletion of *gprC* resulted in delayed mortality whereas deletion of *gprD* attenuated virulence, when administered intranasally, in a neutropenic mouse model of infection, although the mechanism behind this reduction in virulence is unknown (Gehrke *et al.*, 2010). GprK plays a role in carbon sensing, with the null mutant displaying increased germination under carbon starvation conditions and decreased growth on pentose sugars. Although this mutant showed significantly less invasion into A549 cells *in vitro*, there was no significant difference in survival using a wax moth larvae model, thus the role of this receptor in pathogenesis remains unclear (Jung *et al.*, 2016).

The second major glucose sensing mechanism in *S. cerevisiae* uses high and low affinity hexose transporters (HXT), Snf3p and Rgt2p, respectively. These two proteins do not actively transport glucose but rather act as sensors for glucose concentration and transduce signals that regulate the expression of HXT genes appropriate for the glucose environment (Forsberg and Ljungdahl, 2001, Ozcan *et al.*, 1998). In *C. albicans*, there are 20 predicted hexose transporters (*HGT1-20*), one of which encodes an Snf3p/Rgt2p homologue, Hgt4p, which senses glucose and subsequently regulates the expression of 6 *HGT* genes and is partially responsible for filamentation in response to this stimulus (Brown *et al.*, 2006, Fan *et al.*, 2002). Loss of *HGT4* results in an increased median survival of mice when injected intravenously into an immunocompetent systemic model of candidiasis, supporting the hypothesis that sugar sensing is an important aspect of *C. albicans* virulence (Brown *et al.*, 2006). Furthermore, *HGT6* and *HGT12* were highly expressed in a mouse model of oral candidiasis (OPC) when compared to the *in vitro* control condition (Fanning *et al.*, 2012), *HGT7* was significantly expressed in a mouse model of intra-abdominal candidiasis (Cheng *et al.*, 2013) and *HGT2, 4, 7, 12* and *17* were induced during an infection time course experiment in mice with systemic candidiasis (Wenjie *et al.*, 2015). These results indicate that sugar scavenging, sensing and/or transport are important during early and late infection

at various sites of the mammalian host. The exact role played by these *HGT* genes in *C. albicans* virulence remains to be determined.

The most closely related proteins to Snf3p/Rgt2p/Hgt4p in *C. neoformans* are Hxs1 and Hxs2. Transcriptome analysis of *C. neoformans* strains isolated from the cerebrospinal fluid (CSF) of 2 AIDS patients, showed high induction of Hxs1 when compared to the *in vitro* control condition (Chen *et al.*, 2014). Hxs1 was shown to be a high affinity glucose transporter that did not complement the glucose sensing defect of an *S. cerevisiae SNF3/RGT2* mutant, nor did loss of *HXS1* affect expression of hexose transporters, suggesting that it may not act as a glucose sensor (Liu *et al.*, 2013). Deletion of *HXS2* was not possible but a role in glucose sensing is questioned given the low expression of this gene in high and low glucose (Liu *et al.*, 2013). Sugar sensing and uptake systems and their relationship with virulence have not been characterized in *A. fumigatus*, however 17 putative hexose transporter genes have been identified in *A. nidulans* (Wei *et al.*, 2004). Among these 17 hexose transporters, 15 predicted homologues are found in *A. fumigatus*. In summary, significant opportunities exist to better define the roles of sugar sensing and uptake in human fungal virulence.

Carbon catabolite repression (CCR)

In many fungi, carbon source utilization is regulated via a finely-tuned system that allows preferential utilization of glucose and preferred sugars before other available carbon sources. This system is called Carbon Catabolite Repression (CCR) and is regulated in part by the C_2H_2 zinc-finger transcription factor Mig1p/CreA/Mig1, which represses the expression of genes required for catabolism of less preferable carbon sources, as well as gluconeogenic genes, and nutrient acquisition genes in response to carbon starvation (Ruijter and Visser, 1997; Zaragoza *et al.*, 2000; Caza *et al.*, 2016). CCR in *S. cerevisiae*, regulated by the transcriptional repressor Mig1p and the corepressors Tup1p and Ssn6p, and CreA in *A. nidulans* and *Aspergillus niger*, has been studied exhaustively and is reviewed in (Kim *et al.*, 2013, Gancedo, 1998, Klein *et al.*, 1998).

Transcriptional studies of Mig1p and Tup1p in *C. albicans*, indicate that each transcription factor regulates unique sets of genes, in addition to shared genes between both repressors (Murad *et al.*, 2001). Unique Mig1p targets and those shared by Mig1p and Tup1p were largely annotated as carbohydrate uptake and catabolism factors, implicating these repressors in CCR. Furthermore, microarray studies of Mig1p identified this regulator to have predominantly repressing functions, with only 0.8% of measured genes down-regulated upon loss of Mig1p, versus ~12.5% of measured genes whose mRNA levels increased in response to Mig1p loss (Murad *et al.*, 2001).

In *C. neoformans*, Mig1 regulates the expression of amino acid, heme and carboxylic acid biosynthesis genes, TCA cycle and electron transport genes. The loss of Mig1 results in increased sensitivity to oxidative stress, respiratory inhibitors and antifungal drugs. Interestingly, a large number of genes are regulated by Mig1 under iron limitation, suggesting a role for this protein in iron homeostasis or metabolic adaptation to low iron (Caza *et al.*, 2016). Indeed, a relationship of Mig1 with HapX, a bZIP transcription factor required for adaptation to iron limiting and iron excess conditions and virulence in several

fungal pathogens (Jung *et al.*, 2010, Schrettl *et al.*, 2010, Gsaller *et al.*, 2014, Chen *et al.*, 2011, Lopez-Berges *et al.*, 2012), was identified in *C. neoformans* (Caza *et al.*, 2016). Although current studies on Tup1 provide little evidence as to whether Tup1 is involved in CCR (Lee *et al.*, 2007, Lee *et al.*, 2005, Lee *et al.*, 2009), a role for Tup1 in positive regulation of iron homeostasis genes was identified, including *CIG1*, a gene encoding the putative heme binding protein (Lee *et al.*, 2009), that was also identified to be positively regulated by Mig1 (Caza *et al.*, 2016). Although interactions have yet to be identified between Mig1 and Tup1 in *C. neoformans*, a potential mechanistic link between these regulatory proteins in low iron conditions is an intriguing possibility.

In filamentous fungi, the Mig1p homologue, CreA, has been well studied in *A. nidulans* where the presence of glucose results in CreA translocation to the nucleus and subsequent repression of genes encoding enzymes required for the utilization of alternative carbon sources (Kulmburg *et al.*, 1993, Tamayo *et al.*, 2008, Garcia *et al.*, 2004; Brown *et al.*, 2013; Ries *et al.*, 2016). Unlike CCR in yeasts, the Tup1p homologe, RcoA does not play a role in CCR in *A. nidulans* at the level of gene expression, however, loss of RcoA results in changes in nucleosome positioning at some CreA regulated promoters (Hicks *et al.*, 2001, Garcia *et al.*, 2008). Notably, loss of CreA in *A. nidulans* results in a leaky lethal phenotype (Dowzer and Kelly, 1991), resulting in an extremely sick strain. Full lethality of this gene deletion has been reported in several other filamentous fungi including *Fusarium oxysporum* (Jonkers and Rep, 2009), *Penicillium chrysogenum* (Cepeda-García *et al.*, 2014) and *Colletotrichum gloeosporoides* (Bi *et al.*, 2015), whereas in *Neurospora crassa* (Sun and Glass, 2011), *Trichoderma reesei*, and *A. fumigatus* (Beattie *et al.*, 2017), generation of a *creA*-genetic null mutant is viable, underscoring divergence of the role of this transcription factor across fungal species. In *A. fumigatus*, the loss of *creA* results in a carbon and nitrogen-independent growth defect on solid media, with carbon-dependent growth defects in liquid culture. Similar to *C. neoformans*, loss of *creA* also results in increased sensitivity to mitochondrial inhibitors and the antifungal drug, voriconazole. It is unclear if the increased susceptibility to these inhibitors is a direct effect, or a result of increased drug permeability due to altered cell wall composition in the *creA* null mutant. Interestingly, in contrast to *C. albicans*, where the predominant changes in gene expression of *MIG1* indicated a repressor only function for Mig1p, RNA-sequencing of the *A. fumigatus creA* genetic null mutant revealed nearly equal numbers of genes whose transcript levels decreased and increased in glucose medium compared to the wild-type parent (Beattie *et al.*, 2017). While work to characterize the direct targets of CreA is ongoing, these results suggest that CreA may play a role in transcriptional activation in addition to repression in *A. fumigatus* as previously observed in *Trichoderma reesei* (Portnoy *et al.*, 2011) and *A. nidulans* (Mogensen *et al.*, 2006).

The variable essentiality of Mig1/CreA homologues across fungal species suggests functional divergence of this transcription factor. Indeed, primary amino acid sequence alignments of Mig1/CreA between yeasts and filamentous fungi show that only the C_2H_2-zinc finger domain is conserved (Figure 3). Thus, understanding the role of these transcription factors and their interacting partners in each species is a promising approach to better understand and define the metabolic similarities and differences of between fungal species and how these differences impact metabolism and growth *in vivo*.

Additional factors involved in CCR

In *S. cerevisiae*, glucose repression is dependent on the hexokinase, Hxk2p, which is required for phosphorylation of Mig1p by Snf1p (Herrero *et al.*, 1998, Ahuatzi *et al.*, 2007). In *A. fumigatus*, the hexokinases, *hxkA* and to a larger extent *glkA*, appear to also play a role in CCR, as loss of these proteins increase isocitrate lyase (ICL, glyoxylate cycle) activity in the presence of glucose and ethanol (Fleck and Brock, 2010). The expression of *C. albicans HXK2* is regulated in response to glucose, however, null mutants of this gene have yet to be characterized and a role in CCR remains undefined. In *C. neoformans*, the deletion of both hexokinases, *HXK1* and *HXK2*, results in the inability to utilize glucose, however their role in CCR has not yet been investigated. The loss of both hexokinases together, but not single deletions, results in reduced virulence when inoculated intranasally in an immunocompetent mouse model (Price *et al.*, 2011).

The AMP-activated protein kinase Snf1p plays an important role in CCR in *S. cerevisiae* through regulating the cellular localization of Mig1p (De Vit *et al.*, 1997, Ostling and Ronne, 1998). Snf1p belongs to a highly conserved family of serine/threonine protein kinases, with an essential role in mediating the response to glucose abundance/depletion thus regulating cellular energy homeostasis (Mizuno *et al.*, 2015). Regulation of Mig1p by Snf1p has not yet been characterized in *C. albicans*, but a homozygous mutant of Snf1p is lethal (Petter *et al.*, 1997). Characterization of a heterozygous *SNF1* mutant revealed a growth defect, however carbon source utilization was not altered, and the mutant maintained wild-type virulence in an immunocompetent systemic model, despite a hyper-filamentous phenotype *in vitro* (Petter *et al.*, 1997). Similar to *S. cerevisiae*, re-localization of *A. nidulans* CreA to the cytoplasm following transfer to glucose starvation conditions, as well as expression of cellulase and hemicellulase-encoding genes is dependent on SnfA (Brown *et al.*, 2013). However, the role of SnfA in CCR has yet to be fully defined in filamentous human pathogens. In *C. neoformans*, generation of a *SNF1*-null mutant is viable and experimental evidence implicates this kinase in the regulation of CCR (Hu *et al.*, 2008a). Interestingly, Snf1 appears to have divergent roles across serotypes within the species (Hu *et al.*, 2008a) but in all strains, Snf1 is essential for virulence in both pulmonary and systemic immunocompetent models of cryptococcosis (Hu *et al.*, 2008a, Yang *et al.*, 2010).

Studies of CCR in *A. nidulans* also identified three additional factors which regulate glucose repression in this organism: CreB, CreC and CreD (Hynes and Kelly, 1977, Kelly and Hynes, 1977). CreB, a deubiquitination enzyme (Lockington and Kelly, 2001), and CreC, a WD-40 repeat protein (Todd *et al.*, 2000), form a complex that is required for proper glucose repression (Lockington and Kelly, 2002). Mutation of either of these proteins yields strains that have growth defects in both repressing and de-repressing conditions (Hynes and Kelly, 1977, Lockington and Kelly, 2001, Ries *et al.*, 2016). Although CreB has deubiquitination activity, the role of ubiquitin in the regulation of CreA is unclear. Ries and colleagues (Ries *et al.*, 2016) identified an ubiquitin smear on a CreA immunoprecipitation Western blot, however, mass spectrometry to identify post-translational modifications on CreA found several phosphorylated peptides, but no ubiquitinylated peptides in repressing or de-repressing conditions (Alam *et al.*, 2016). CreD, on the other hand, is an arrestin and PY motif containing protein that appears to regulate CCR through opposing the action of the

CreB/CreC complex. Yeast two-hybrid studies of CreD identified a physical interaction between CreD and the ubiquitin ligase, HulA, suggesting that this protein may be involved in ubiquitination of targets, potentially sharing targets with the CreB/CreC complex (Boase and Kelly, 2004). Homologues of CreB, CreC and CreD are all present in *A. fumigatus* but a role of these proteins in virulence has not yet been determined. Homologues of these three proteins are also present in *C. albicans* and include the ubiquitin hydrolase Upb13p (CreB orthologue) with a predicted role in biofilm formation (Nett *et al.*, 2009; Bonhomme *et al.*, 2011); the alpha subunit of the COPI (coat protein) vesicle coatomer complex (CreC orthologue) which functions in vesicle and retrograde vesicle-mediated transport between the ER (endoplasmic reticulum) and the Golgi (Bonhomme *et al.*, 2011) and lastly, the membrane protein Rod1p (CreD orthologue) which has a role in drug tolerance and is under the transcriptional control of Rgt1p, which is also involved in the regulation glucose transporter-encoding genes (Sexton *et al.*, 2007). A recent study described the involvement of *A. oryzae* CreD in glucose-induced endocytosis of a maltose transporter, whose expression is regulated by CCR, through working as an adaptor protein for HulA, required for the ubiquitination of target plasma membrane transporters and subsequent endocytosis (Tanaka *et al.*, 2017). It is therefore an intriguing possibility that the role of CreB, CreC and CreD may be one in CCR-related vesicle transport, responding to a change in available nutrients, rather than directly targeting CreA. No homologues of these three proteins have been identified in *C. neoformans*.

Role of CCR in virulence

Progress towards understanding the utilization of potential carbon sources during an infection has been made using transcriptional studies of C. *albicans, A. fumigatus and C. neoformans* exposed to immune cells or in infection models (Fradin *et al.*, 2005, Lorenz *et al.*, 2004, Barelle *et al.*, 2006, Chen *et al.*, 2014). Similarities between the response of these fungi to phagocytosis include the induction of metabolic pathways required during carbon starvation, including gluconeogenesis, fatty acid metabolism and the glyoxylate shunt, many of which are targets of the CCR system in these fungi. However, phagocytosis represents only one host niche, and during infection, occupation of several niches is likely required for full pathogenesis. This is demonstrated by reporter fusion studies, where Barelle and colleagues (2006) reported the upregulation of the glyoxylate cycle genes in phagocytosed cells, however, the population of cells in the kidney during systemic infection were largely expressing glycolytic genes and some fungal cells even expressed gluconeogenic and glyoxylate cycle genes. The aforementioned studies indicate that fungal cells experience a variety of micro-environmental conditions *in vivo*, and the ability to adapt to and thrive under several carbon conditions is critical for virulence (Barelle *et al.*, 2006). Furthermore, characterisation of gene expression in fungal strains isolated directly from human subjects, as has recently be done for 2 *C. neoformans* isolates extracted from the CSF of two AIDS patients (Chen *et al.*, 2014), can also provide novel insights into infection-related processes. Gene expression of *C. neoformans* in human CSF was more similar to the *in vitro* YPD control condition than in *ex vivo* CSF, an observation that was predicted to be due to varying nutrient availability, pH and epigenetic changes that occur specifically in the human host (Chen *et al.*, 2014). Such studies are valuable as they characterise the genetic response of fungal pathogens that are metabolically active during human host infection, and do not rely

on animal models or *in vitro* conditions that simulate mammalian environments, therefore providing insight into *in vivo* infection processes in the human host.

These studies highlight the potential for differential metabolic requirements across fungal pathogens, likely dependent on the host niche occupied by each species and the models used to test each mutant. Further support for niche specific metabolic requirements comes from Hu and colleagues (Hu *et al.*, 2008a), who reported differential expression of several core carbon metabolism genes in *C. neoformans*, including the high affinity glucose transporter *HXT1* and phosphoenolpyruvate carboxykinase *PCK1*, which were highly expressed early in pulmonary infection, but not in CSF infection, suggesting low glucose availability in the lungs early in infection. The same study identified the upregulation of carbon starvation and alternative carbon utilization pathways, such as beta-oxidation, the glyoxylate shunt and the utilization and production of acetyl-coA (Hu *et al.*, 2008a, Steen *et al.*, 2003). Furthermore, deletion of the high-affinity glucose transporter *HXS1*, required for growth in low glucose, results in attenuated virulence of *C. neoformans* in a pulmonary model of cryptococcosis (Liu *et al.*, 2013).

Although these clues to *in vivo* carbon source utilization have identified expression of targets of carbon catabolite repression, Mig1p/Mig1 is dispensable for virulence (as measured by murine mortality) in an immunocompetent systemic model of *C. albicans* and an immunocompetent pulmonary model of *C. neoformans*, suggesting that at least in the models tested, the global de-repression of carbon catabolite genes is not detrimental to growth within the host or the expression of required virulence factors (Zaragoza *et al.*, 2000, Caza *et al.*, 2016). Interestingly, although *MIG1* deletion had no effect on virulence in a murine inhalation model of cryptococcosis, fungal burden in the blood of *MIG1* deletion inoculated animals was significantly higher than wild type inoculated animals at the time of death (Caza *et al.*, 2016). This suggests that although mitochondrial metabolism in this mutant is perturbed, the metabolic state provides a growth or survival advantage in this niche (Caza *et al.*, 2016).

In contrast to the pathogenic yeasts, *A. fumigatus* CreA plays a role in virulence in a host-specific manner. The absence of CreA results in a significant reduction in virulence of *A. fumigatus* in a triamcinolone model of IPA highlighted by qualitative differences in immune effector cell function, however, in a neutropenic model of IPA highlighted by quantitative differences in leukocyte populations, the *creA*-null mutant causes 100% mortality with a one-day increase in median survival compared to wild type. Notably, the defect in virulence in the triamcinolone model is not due to a defect in initiation of infection, as both wild type and the *creA* null mutant germinate and grow to the same extent over the first 48 hours of infection. Thus, it is fitness in the established infection site microenvironment where the *creA* null mutant fails (Beattie *et al.*, 2017). In support of this, established biofilms of the *creA* null mutant are unable to proliferate in low oxygen conditions, a feature of fungal lesions that has been characterized in the triamcinolone model of IPA (Grahl *et al.*, 2011, Beattie *et al.*, 2017). Thus the role of this transcription factor in virulence depends on the context of the host immune status and microenvironmental conditions, and is not required for *in vivo* growth per se, but for adaptation to the established infection site microenvironment. CreA was thus termed a *disease progression factor (DPF)* for genes

dispensable for infection initiation but critical for infection persistence and progression. It is hypothesized that other key regulators of fungal and host metabolism are likely DPFs given the profound changes that occur in nutrient availability during the course of microbial infections.

In addition, however, the implication of several CCR regulated pathways *in vivo* and the apparent lack of a role for yeast Mig1p during infection using standard genetic null mutants raises an interesting question as to whether a genetic null mutant is the best way to assess the role of a transcriptional repressor *in vivo*. If de-repression of alternative carbon source utilization *in vivo* is required for growth, perhaps a constitutively repressing Mig1p/CreA mutant may yield additional insights to the role of CCR *in vivo*. Studies in *S. cerevisiae* have identified a phosphorylation site on serine 311, that when mutated, results in constitutive repression of target genes (Ahuatzi *et al.*, 2007). While it is unclear whether regulatory mechanism of Mig1p homologues in pathogenic fungi are conserved, creation of these types of mutants may give more insight to the role of CCR and the importance of metabolism of alternative carbon sources in various infection models.

The importance of understanding carbon source utilization *in vivo* is exemplified by studies that show the carbon source and nutrient environment influences virulence determinants and efficacy of drug treatments. Ene and colleagues showed that growth on the physiologically relevant carbon source, lactate, changes fungal cell wall architecture and structure, which affects the physical properties and results in increased resistance to osmotic stress, cell wall perturbing agents and antifungal drugs, a phenomenon that occurs across *Candida* species (Ene *et al.*, 2012). Further, cells grown on lactate or amino acids were more virulent in an intravenous model of candidiasis (Ene *et al.*, 2012), and lactate-grown cells were phagocytosed less efficiently than glucose-grown cells, and increased IL-10 and IL-17 immune response from PBMCs (Ene *et al.*, 2013). Similar observations in *A. fumigatus* have revealed altered cell wall composition based on growth medium, where growth on yeast extract resulted in reduced beta- and alpha-glucan levels and increased chitin when compared to cells grown in RPMI. These differences in cell wall composition translate to differences to an increased minimal effective concentration (MEC) of caspofungin in RPMI when compared to yeast extract, and increased efficacy of combinatorial treatment with caspofungin and nikkomycin Z in RPMI versus yeast extract (Clavaud *et al.*, 2012). Furthermore, for *C. albicans*, glucose, as well as galactose, fructose and sucrose, stimulate hyphal growth, a morphological feature that is considered to be a major virulence factor of this organism (Hudson *et al.*, 2004, Maidan *et al.*, 2005b). These changes in virulence attributes and drug resistance are important to understand, especially from the perspective of treatment, given that lab tests for drug susceptibility are performed in RPMI, a glucose-rich media, which given the above results, may not be an accurate reflection of the susceptibilities of strains within a patient. Therefore, understanding the metabolic state of cells within a host not only opens up the possibility of novel metabolic drug targets, but may also help in developing drug susceptibility testing that is more reflective of the *in vivo* status of the fungus. Table 1 lists all aforementioned CCR-related factors and their importance in virulence.

Nitrogen catabolite metabolism in human pathogenic fungi

Ammonium and glutamine sensing and uptake

The preferred nitrogen sources for most microorganisms are ammonium and glutamine as they are easily assimilated and readily used for protein synthesis (Wong *et al.*, 2008). Ammonium and glutamine metabolism are intrinsically linked, with glutamine being synthesised from ammonium and glutamate by the enzyme glutamine synthetase (Margelis *et al.*, 2001). Fungi have evolved several sophisticated nitrogen sensory and uptake systems which allow the cell to scavenge the preferred nitrogen sources, even when present in limiting concentrations. The genome of *C. albicans* encodes 2 ammonium permeases *MEP1* and *MEP2*, that allow growth under low ammonium concentrations, with Mep2p shown to be crucial for pseudohyphal growth induction through activating downstream mitogen activated protein (MAP) kinase and cAMP-dependent pathways (Biswas and Morschhäuser, 2005; Dabas *et al.*, 2009). Homologues of Mep1p and Mep2p are encoded in the genome of *A. fumigatus* (MeaA, Afu1g10930) but have not been characterised to date in this fungus. Similar to *C. albicans*, low concentrations of extracellular ammonium induce pseudohyphal growth in *C. neoformans*, a process that relies on the two ammonium permeases Amt1 and Amt2 (Lee *et al.*, 2012). Neither Amt1 nor Amt2 were required for *C. neoformans* pathogenicity when immunocompetent mice were infected by nasal inhalation (Rutherford *et al.*, 2008).

In *C. albicans*, the amino acid sensor Csy1p was shown to sense and induce the transport of glutamine (Brega *et al.*, 2004). Csy1p plays an important role in the induction of pseudohyphal growth in serum-based media (Brega *et al.*, 2004) and together with the general amino acid permease (AAP) and major nitrogen source sensor Gap2p (Kraidlova *et al.*, 2011) activates the PKA (protein kinase A) pathway which is important for virulence in pathogenic fungi (Donaton *et al.*, 2003; Kraidlova *et al.*, 2016). A direct role of the homozygous *CSY1* deletion strain in virulence has not been published (Brega *et al.*, 2004). The genome of *A. fumigatus* encodes several AAPs, including the orthologue of Gap2p (Afu7g04290), but none have been characterized so far and their role in virulence remains unknown (Sugui *et al.*, 2008; Morton *et al.*, 2011; Gehrke *et al.*, 2010; Oosthuizen *et al.*, 2011). Similar to *A. fumigatus*, the genome of *C. neoformans* encodes 10 AAPs whose expression is temperature- and nitrogen source-dependent, with Aap4 and Aap5 (both transporting glutamine) being important for growth during high temperature, oxidative stress and *in vivo* virulence in an immunocompetent murine model of pulmonary cryptococcosis (Fernandes *et al.*, 2015; Cruz Martho *et al.*, 2016).

The main nitrogen nutrient sensor, and regulator of downstream signalling pathways is the target of rapamycin (TOR) pathway (extensively reviewed in Rohde *et al.*, 2008). The TOR pathway consists of one or two (depending on the fungal species) Tor protein kinases which are activated by amino acid-, including glutamine, derived signals and regulate a wide variety of cellular processes (Crespo *et al.*, 2002; De Virgilio *et al.*, 2006; Su *et al.*, 2012; Staschke *et al.*, 2010). The genomes of *C. albicans, A. fumigatus* and *C. neoformans* encode one TOR kinase which is essential for viability. In *C. albicans*, Tor1p controls NCR, the expression of Gcn4p, morphogenesis, cell adhesion, and condition-specific biofilm

formation, whereas in *A. fumigatus*, amino acid transport and metabolism are under the regulatory control of TorA (Bastidas *et al.*, 2009; Baldin *et al.*, 2015; Cruz *et al.*, 1999; Lee et al., 2012). Deletion of the *C. albicans* gene *SIT4*, homologue of the *S. cerevisiae* PP2A-like protein phosphatase Sit4p which mediates NCR repression by TORC1, significantly affected yeast and hyphal growth and resulted in a strain with attenuated virulence in an immunocompetent mouse model of systemic candidiasis (Lee *et al.*, 2004). In *C. albicans*, Tor1p-mediated signalling is controlled by the small GTPase Rhb1p and the GTPase-activating protein Tsc2p, both of which are thought to control the expression of the ammonium permease Mep2p (Tsao *et al.*, 2009). Rhb1p homozygous deletion mutants are viable although a role in infection of this strain has not been investigated yet. Similar to *C. albicans*, the *A. fumigatus* PP2A-like protein phosphatase SitA, was shown to be important for the cell wall integrity (CWI) pathway, adhesion and the deletion of the corresponding gene resulted in an avirulent strain when administered by nasal instillation in a neutropenic mouse model (Bom *et al.*, 2015). Similarly, deletion of the GTPase RhbA, homologue of *C. albicans* Rhb1p, resulted in a strain with attenuated virulence when in a neutropenic mouse of pulmonary aspergillosis (Panepinto *et al.*, 2003). In *C. neoformans*, Sin1, the orthologue of the *S. cerevisiae* TORC2 complex component Avo1p, and Ypk1, which is involved in the TOR-dependent phosphorylation of ribosomal proteins in *S. cerevisiae*, were shown to be important for virulence in an immunocompetent murine model of infection when administered via tail vein injection (Lee *et al.*, 2012). Additional TOR-mediated, nitrogen metabolism-related signalling processes have not been studied in *C. neoformans*.

Nitrogen catabolite repression (NCR)

The preferential utilisation of easily assimilated nitrogen sources, such as ammonium and glutamine, is known as NCR. In *A. nidulans*, NCR is mediated by the co-repressor NmrA, with the deletion of the corresponding gene resulting in the partial de-repression of genes encoding enzymes required for the utilisation of alternative nitrogen sources such as asparagine and urea in the presence of favoured nitrogen sources (Andrianopoulos *et al.*, 1998). The genome of *A. fumigatus* encodes a homologue of *A. nidulans* NmrA (Afu5g02920) although it has not been characterised and a role in virulence has not been studied to date. Homologues of NmrA do not appear to present in the genomes of *C. albicans* and *C. neoformans*.

Upon the depletion of the preferred nitrogen sources ammonium and glutamine, the fungus switches from nitrogen anabolism to catabolism by inducing the expression of catabolic genes required for the scavenging and utilisation of energetically less favoured nitrogen sources (Tudzynski, 2014). The GATA-type transcription factor Gat1p/AreA/Gat1 induces the expression of catabolic genes in *C. albicans, A. fumigatus* and *C. neoformans*. The functions as well as the role in virulence of Gat1p/AreA/Gat1 diverge in all three fungal species. Deletion of *GAT1* in *C. albicans* still allowed the use of a number of nitrogen sources except for isoleucine, tyrosine and tryptophan (Limjindaporn *et al.*, 2003). The genome of *C. albicans* encodes a second GATA-type transcription factor Gln3p, which shares overlapping functions with Gat1p, regulating the expression of genes encoding enzymes involved in nitrogen metabolism and ammonium or amino acid uptake (Liao *et al.*, 2008). Both transcription factors have been shown to be crucial for virulence in an

immunocompetent murine model of systemic disseminated candidiasis (Limjindaporn *et al.*, 2003; Liao *et al.*, 2008), which may be due to the inability of the homozygous deletion mutants to induce filamentation in a nitrogen source-dependent manner (Liao *et al.*, 2008). Deletion of *areA* in *A. nidulans* and *A. fumigatus* abolishes growth on a wide range of nitrogen sources except for ammonium and glutamine (Langdon *et al.*, 1995; Hensel *et al.*, 1998). AreA was shown to contribute to, but not be essential for *A. fumigatus* virulence in a neutropenic murine model of pulmonary aspergillosis, with the deletion mutants presenting a delayed growth phenotype during infection (Hensel *et al.*, 1998). In *C. neoformans*, deletion of *GAT1* resulted in severely reduced growth on most amino acids (except arginine and proline), urea and ammonium (Kmetzsch *et al.*, 2011). Knockout of *GAT1* in *C. neoformans* was shown to be important for the production of capsular glucuronoxylomannan, but did not affect virulence in an immunocompetent murine model of pulmonary cryptococcosis (Kmetzsch *et al.*, 2011; McClelland *et al.*, 2005).

NCR is subject to negative regulation and in *S. cerevisiae*, this is mediated by the two GATA transcription factors Dal80p and Gzf3p. Both regulators are predicted to antagonise the functions of Gln3p and Gat1p by competing for the same binding site in NCR-regulated genes (Coffman *et al.*, 1997; Svetlov and Cooper, 1998; for an extensive review please refer to Magasanik and Kaiser, 2002). The genome of *C. albicans* encodes only one homologue of *S. cerevisiae* Gzf3p and Dal80p, termed *GZF3*, whose deletion resulted in altered colony morphology at 30°C and 37°C (Homann *et al.*, 2009). A role for this transcription factor in virulence has not been investigated yet. In *A. nidulans*, the homologue of Dal80p is the GATA zinc finger transcription factor AreB, which was predicted to antagonise the activation of AreA under nitrogen-limiting or – starvation conditions (Wong *et al.*, 2009). The mechanistic nature underlying AreB-mediated regulation of AreA is currently unknown. In addition, in the presence of ammonium and glutamine, NmrA interacts with AreA, preventing the induction of genes required for the utilisation of non-preferred nitrogen sources; whereas in the absence of ammonium and glutamine, NmrA dissociates from AreA, allowing nitrogen catabolite gene activation (Andrianopoulos *et al.*, 1998; Han *et al.*, 2016). Transcription of *nmrA* and *areA* is inversely regulated, with both genes responding to available nitrogen sources in an opposite manner, whereas overexpression of *nmrA* prevented AreA function irrespective of the nitrogen source (Wong *et al.*, 2007). Furthermore, *nmrA* transcription was shown to be dependent on MeaB, a bZIP DNA-binding protein, with transcriptional expression patterns of both *nmrA* and *meaB* mirroring each other (Wong *et al.*, 2007). The expression of *meaB* was repressed under nitrogen starvation conditions and induced in the presence of preferred and alternative carbon sources (Wong *et al.*, 2007). In *A. fumigatus*, the homologue of MeaB (Afu3g10930) has not been characterised. In *C. neoformans*, additional regulatory proteins involved in NCR have not been described and protein BLAST analysis of the aforementioned *S. cerevisiae, C. albicans* and *A. nidulans* factors against the *C. neoformans* genome database did not generate any significant hits.

Similar to CCR discussed above, the aforementioned studies highlight substantial differences in the regulation of NCR and the role it plays in establishing and maintaining an infection in all three human fungal pathogens. In *Aspergillus spp.*, there appears to be a clear division between the utilisation of preferred and non-preferred nitrogen sources, with both

processes being regulated by two independent factors which also control the function of each other (Adrianopolous *et al.*, 1998). In *C. albicans*, two independent transcription factors also regulate nitrogen metabolism, although a clear division between NCR and NC induction, as seen in *Aspergillus spp.*, is not observed. Both transcription factors rather have overlapping and specific functions regulating genes encoding enzymes required for the utilisation of a wide range of nitrogen sources, thereby creating a complex regulatory network underlying nitrogen metabolism in this fungus (Limjindaporn *et al.*, 2003; Liao *et al.*, 2008). In *C. neoformans*, only one transcription factor involved in NCR has been characterised that is required for growth on both preferred and non-preferred (with the exception of proline and arginine) nitrogen sources (Kmetzsch *et al.*, 2011). It remains to be determined how Gat1 regulates the utilisation of different nitrogen sources and whether a second NCR transcription factor exists that could be involved in these processes.

Additional factors involved in ammonium and glutamine acquisition

In *C. neoformans*, the enzyme urease, which converts urea into ammonium, is an important virulence factor, that is thought to contribute, together with additional factors, to the transmigration of the blood-brain-barrier (BBB) (Shi *et al.*, 2010). Indeed, disruption of the *C. neoformans* urease-encoding gene resulted in attenuated virulence in both an immunocompetent murine inhalation and intravenous infection model of cryptococcosis (Cox *et al.*, 2000). The exact mechanism by which urease production enables dissemination into the brain is not known but ammonia production could increase adherence or toxicity to capillary endothelial cells (Olszewski *et al.*, 2004; Shi *et al.*, 2010). The genomes of both *C. albicans* and *A. fumigatus* also encode ureases but a role for these enzymes in infection has not been determined.

A key feature and major virulence factor of pathogenic fungi is their ability to secrete different classes of proteases which degrade proteins, therefore releasing peptides and/or amino acids which can subsequently be taken up and metabolised (Monod *et al.*, 2002) when extracellular nitrogen sources are scarce. In *C. albicans*, ten aspartic proteinases (Sap1p-Sap10p) are secreted in an infection stage-dependent manner and have been shown to mediate various virulence attributes such as adhesion, biofilm formation and host tissue penetration (Naglik *et al.*, 2003; 2004; 2008). Furthermore, they present various immunomodulatory properties, thus allowing evasion of the host immune responses (Gropp *et al.*, 2009; Bras *et al.*, 2012; Wu *et al.*, 2013). Single deletions of *SAP1, SAP2* and *SAP3* resulted in attenuated virulence when injected intravenously into immunocompetent mice (Hube *et al.*, 1997) whereas the simultaneous deletion of *SAP4, SAP5* and *SAP6*, led to reduced growth in protein-rich medium and attenuated virulence in immunocompetent mouse and guinea pig models of systemic candidiasis (Sanglard *et al.*, 1997). Sap8p is essential for regulating CWI and biofilm formation through the proteolytic cleavage of Msb2p, a sensor of the Cek1p-MAPK pathway, a factor that is important for virulence (Puri *et al.*, 2012).

The genome of *A. fumigatus* is predicted to encode at least 99 secreted proteases (Machida *et al.*, 2005; Nierman *et al.*, 2005) of which different mixtures are secreted in a nitrogen source-dependent manner (Farnell *et al.*, 2012). Protease secretion is under the control of

NCR mediated by the transcriptional regulator, PrtT, which was shown to not be essential for virulence when neutropenic and cortisone acetate-treated mice models of pulmonary aspergillosis (Bergmann *et al.*, 2009; Sharon *et al.*, 2009). Similar to *C. albicans*, proteases modulate host immune responses (Behnsen *et al.*, 2010) and are thought to contribute to *A. fumigatus* virulence by facilitating host tissue degradation and invasion (Gautam *et al.*, 2006; Balenga *et al.*, 2015; Kogan *et al.*, 2004). Deletion of the alkaline serine protease *alp1/aspf13* led to significantly reduced virulence in nasally infected neutropenic mice (Kolatukuddy *et al.*, 1993), although a more recent study showed that disruption of *alp1* had no significant effect on virulence, when the strain was administered intranasally, in a cortisone acetate mouse model (Behnsen *et al.*, 2010). This discrepancy may be due to the use of different mouse models or fungal strains. A high degree of redundancy is likely to exist amongst these secreted proteases as no other secreted protease has been shown to play an important role in virulence in *A. fumigatus*.

Proteases secreted by *C. neoformans* also have immunomodulatory properties (Chen *et al.*, 1996) and some have been found to play a role in modulating cell wall integrity (CWI) pathway components that are important for virulence (Bien *et al.*, 2009). The metalloproteinase Mpr1 was shown to be required for establishing an infection in the central nervous system (CNS) by promoting migration of *C. neoformans* across the brain endothelium (Vu *et al.*, 2014). Deletion of *MPR1* resulted in attenuated virulence due to reduced brain fungal burden in an immunocompetent mouse model of pulmonary and systemic cryptococcosis (Vu *et al.*, 2014). Similarly, *C. neoformans* serine proteases are thought to increase the permeability of the blood brain barrier (BBB) therefore facilitating CNS invasion (Xu *et al.*, 2014).

Role of NCR in virulence

The utilisation of preferred nitrogen sources affects various virulence determinants in human pathogenic fungi. In *C. albicans*, limiting ammonium levels induce filamentous growth, a prominent virulence factor (Biswas and Morschhäuser, 2005). Furthermore, several amino acids, including glutamine, promote alkalinization of the extracellular environment, triggering hyphal morphogenesis and ensuring fungal survival in environments with a range of different pHs which is predicted to be important during infection establishment (Vylkova *et al.*, 2011). In *C. neoformans*, growth in the presence of ammonium results in reduced capsule size but increases cellular melanin content (Kronstad *et al.*, 2011) that has been determined to be an important virulence factor (Jacobson, 2000). In *A. fumigatus* however, a relationship between the utilisation of preferred and non-preferred nitrogen sources and the elaboration of virulence factors has not been extensively studied.

In general, carbon metabolism has received more attention than nitrogen metabolism, although several studies investigating gene expression in pathogenic fungi during early pulmonary infection showed induction of genes required for amino acid biosynthesis and transport, thereby suggesting nitrogen starvation is an important stress response faced by fungi during infection establishment (McDonagh *et al.*, 2008; Hu et al., 2008). Further characterisation of nitrogen metabolism in *C. albicans, A. fumigatus* and *C. neoformans* during later infection time points and in different host niches is therefore required in order to

further dissect the role of nitrogen acquisition during *in vivo* host infection and its relevance for fungal virulence determinants. NCR is intrinsically embedded in a complex regulatory network that finely tunes nitrogen metabolism, adapting the fungal cells to different types and concentrations of nitrogen sources, influencing virulence factor and potentially disease outcome. Table 2 summarises all aforemention factors that are important for NCR as well as their role in virulence in all three human pathogenic fungi.

Future perspectives

The aforementioned studies highlight the importance of carbon and nitrogen metabolism mechanisms in fungal pathogenicity and virulence as they are required for infection establishment and progression and modulating production and activity of virulence factors such as fungal cell wall structure/content and melanin, or in the case of yeast, phenotypic dimorphism. The current understanding on when and where specific pathways are required during an infection and their mechanistic nature, presents a major knowledge gap and detailed characterisation of these pathways in *in vivo*-relevant conditions is expected to lead to the identification of unique targets or strategies for anti-fungal drug development. In this respect, fungal species-specific niches and mechanisms need to be studied, preferably in multiple clinically relevant animal models, as the severity and degree of an infection varies with the underlying immune system disturbance. In contrast to *A. fumigatus, C. albicans* is often isolated from blood cultures, supporting crucial differences in infection exerted by different fungal pathogens. The role of CCR and NCR in this process remains unclear as insufficient data exists, but systemic dissemination is likely to rely on additional factors such as fungal morphology and the progression and/or the development of the immune status of the patient. *A. fumigatus* rapidly germinates in the lung airways into hyphae that may be more difficult to disseminate via the blood stream when compared to yeast cells. Systemic dissemination of *A. fumigatus* is a rare event, that is not seen in mice models and typically happens very late in affected human patients. Nevertheless, comparative studies with so called non-pathogens may be revealing as shown for differences in metabolic flexibility between *S. cerevisiae* and *C. albicans* due to the presence/absence of ubiquitination sites on enzymes required for the utilisation of alternative carbon sources (Sandai *et al.*, 2012).

Acknowledgments

We would like to thank the Conselho Nacional de Desenvolvimento Científico e Tecnológico (CNPq) and the Fundação de Amparo à Pesquisa do Estado de São Paulo (FAPESP) for providing financial support. SRB was supported, in part, by the National Institute of General Medical Sciences of the National Institutes of Health under Award Numbers T32GM008704 and T32AI007519. RAC holds an Investigator in the Pathogenesis of Infectious Diseases Award supported by the Burroughs Wellcome Fund (BWF) and is also supported by a National Institute of Allergy and Infectious Diseases (NIAID) award 2R01AI081838, National Institute of General Medicine Sciences (NIGMS) award P30GM106394 (Bruce Stanton, principal investigator), and a Cystic Fibrosis Foundation award (Bruce Stanton, principal investigator). The authors declare that no conflict of interest exists in this study. The funders had no role in study design, data collection and analysis, decision to publish, or preparation of the manuscript.

References

Abad A, Fernández-Molina JV, Bikandi J, Ramírez A, Margareto J, Sendino J, et al. What makes *Aspergillus fumigatus* a successful pathogen? Genes and molecules involved in invasive aspergillosis. Rev Iberoam Micol. 2010; 27:155–182. [PubMed: 20974273]

Ahuatzi D, Riera A, Palaez R, Herrero P, Moreno F. Hxk2 regulates the phosphorylation state of Mig1 and therefore its nucleocytoplasmic distribution. J Biol Chem. 2007; 282:4485–4493. [PubMed: 17178716]

Alam MA, Kamlangdee N, Kelly JM. The CreB deubiquitinating enzyme does not directly target the CreA repressor protein in Aspergillus nidulans. Curr Genet. 2016; 63:647–667. [PubMed: 27878624]

Andrianopoulos A, Kourambas S, Sharp JA, Davis MA, Hynes MJ. Characterization of the *Aspergillus nidulans nmrA* gene involved in nitrogen metabolite repression. J Bacteriol. 1998; 180:1973–1977. [PubMed: 9537404]

Ament W, Huizenga JR, Mook GA, Gips CH, Verkerke GJ. Lactate and ammonia concentration in blood and sweat during incremental cycle ergometer exercise. Int J Sports Med. 1997; 18:35–39. [PubMed: 9059903]

Baker EH, Wood DM, Brennan AL, Clark N, Baines DL, Philips BJ. Hyperglycaemia and pulmonary infection. Proc Nutr Soc. 2006; 65:227–235. [PubMed: 16923307]

Baldin C, Valiante V, Krüger T, Schafferer L, Haas H, Kniemeyer O, Brakhage AA. Comparative proteomics of a tor inducible *Aspergillus fumigatus* mutant reveals involvement of the Tor kinase in iron regulation. Proteomics. 2015; 15:2230–2243. [PubMed: 25728394]

Balenga NA, Klichinsky M, Xie Z, Chan EC, Zhao M, Jude J, et al. A fungal protease allergen provokes airway hyperresponsiveness in asthma. Nat Commun. 2015; 6:6763. [PubMed: 25865874]

Barelle CJ, Priest CL, Maccallum DM, Gow NA, Odds FC, Brown AJ. Niche-specific regulation of central metabolic pathways in a fungal pathogen. Cell Microbiol. 2006; 8:961–971. [PubMed: 16681837]

Bastidas RJ, Heitman J, Cardenas ME. The protein kinase Tor1 regulates adhesin gene expression in *Candida albicans*. PLoS Pathog. 2009; 5:e1000294. [PubMed: 19197361]

Beattie SR, Mark KMK, Thammahong A, Ries LNA, Dhingra S, Caffrey-Carr AK, et al. Filamentous fungal carbon catabolite repression supports metabolic plasticity and stress responses essential for disease progression. PLoS Pathog. 2017; 13:e1006340. [PubMed: 28423062]

Behnsen J, Lessing F, Schindler S, Wartenberg D, Jacobsen ID, Thoen M, Zipfel PF, Brakhage AA. Secreted *Aspergillus fumigatus* protease Alp1 degrades human complement proteins C3, C4, and C5. Infect Immun. 2010; 78:3585–3594. [PubMed: 20498262]

Bergmann A, Hartmann T, Cairns T, Bignell EM, Krappmann S. A regulator of *Aspergillus fumigatus* extracellular proteolytic activity is dispensable for virulence. Infect Immun. 2009; 77:4041–4050. [PubMed: 19564390]

Bi F, Barad S, Ment D, Luria N, Dubey A, Casado V, et al. Carbon regulation of environmental pH by secreted small molecules that modulate pathogenicity in phytopathogenic fungi. Molecular Plant Pathology. 2015 n/a-n/a.

Bien CM, Chang YC, Nes WD, Kwon-Chung KJ, Espenshade PJ. *Cryptococcus neoformans* Site-2 protease is required for virulence and survival in the presence of azole drugs. Mol Microbiol. 2009; 74:672–690. [PubMed: 19818023]

Biswas K, Morschhäuser J. The Mep2p ammonium permease controls nitrogen starvation-induced filamentous growth in *Candida albicans*. Mol Microbiol. 2005; 56:649–669. [PubMed: 15819622]

Boase NA, Kelly JM. A role for creD, a carbon catabolite repression gene from *Aspergillus nidulans*, in ubiquitination. Mol Microbiol. 2004; 53:929–940. [PubMed: 15255903]

Bom VL, de Castro PA, Winkelströter LK, Marine M, Hori JI, Ramalho LN, et al. The *Aspergillus fumigatus sitA* Phosphatase Homologue Is Important for Adhesion, Cell Wall Integrity, Biofilm Formation, and Virulence. Eukaryot Cell. 2015; 14:728–744. [PubMed: 25911225]

Bonhomme J, Chauvel M, Goyard S, Roux P, Rossignol T, d'Enfert C. Contribution of the glycolytic flux and hypoxia adaptation to efficient biofilm formation by *Candida albicans*. Mol Microbiol. 2011; 80:995–1013. [PubMed: 21414038]

Borregaard N, Herlin T. Energy metabolism of human neutrophils during phagocytosis. J Clin Invest. 1982; 70:550–557. [PubMed: 7107894]

Bras G, Bochenska O, Rapala-Kozik M, Guevara-Lora I, Faussner A, Kozik A. Extracellular aspartic protease *SAP2* of *Candida albicans* yeast cleaves human kininogens and releases proinflammatory

peptides, Met-Lys-bradykinin and des-Arg(9)-Met-Lys-bradykinin. Biol Chem. 2012; 393:829–839. [PubMed: 22944684]

Brega E, Zufferey R, Mamoun CB. *Candida albicans* Csy1p is a nutrient sensor important for activation of amino acid uptake and hyphal morphogenesis. Eukaryot Cell. 2004; 3:135–143. [PubMed: 14871944]

Brosnan JT. Interorgan amino acid transport and its regulation. J Nutr. 2003; 133:2068S–2072S. [PubMed: 12771367]

Brown NA, de Gouvea PF, Krohn NG, Savoldi M, Goldman GH. Functional characterisation of the non-essential protein kinases and phosphatases regulating *Aspergillus nidulans* hydrolytic enzyme production. Biotechnol Biofuels. 2013; 6:91. [PubMed: 23800192]

Brown V, Sexton JA, Johnston M. A glucose sensor in *Candida albicans*. Eukaryot Cell. 2006; 5:1726–1737. [PubMed: 17030998]

Caza M, Hu G, Price M, Perfect JR, Kronstad JW. The Zinc Finger Protein Mig1 Regulates Mitochondrial Function and Azole Drug Susceptibility in the Pathogenic Fungus *Cryptococcus neoformans*. mSphere. 2016; 1

Cepeda-García C, Domínguez-Santos R, García-Rico RO, García-Estrada C, Cajiao A, Fierro F, et al. Direct involvement of the CreA transcription factor in penicillin biosynthesis and expression of the *pcbAB* gene in *Penicillium chrysogenum*. Applied Microbiology and Biotechnology. 2014; 98:7113–7124. [PubMed: 24818689]

Chen LC, Blank ES, Casadevall A. Extracellular proteinase activity of *Cryptococcus neoformans*. Clin Diagn Lab Immunol. 1996; 3:570–574. [PubMed: 8877137]

Chen C, Pande K, French SD, Tuch BB, Noble SM. An iron homeostasis regulatory circuit with reciprocal roles in *Candida albicans* commensalism and pathogenesis. Cell Host Microbe. 2011; 10:118–135. [PubMed: 21843869]

Chen Y, Toffaletti DL, Tenor JL, Litvintseva AP, Fang C, Mitchel TG, et al. The *Cryptococcus neoformans* transcriptome at the site of human meningitis. MBio. 2014; 5:e01087–13. [PubMed: 24496797]

Cheng S, Clancy CJ, Wenjie X, Schneider F, Hao B, Mitchell AP, et al. Profiling of *Candida albicans* gene expression during intra-abdominal candidiasis identifies biologic processes involved in pathogenesis. J Infect Dis. 2013; 208:1529–1537. [PubMed: 24006479]

Clavaud C, Beauvais A, Barbin L, Munier-Lehmann H, Latge JP. The composition of the culture medium influences the beta-1,3-glucan metabolism of *Aspergillus fumigatus* and the antifungal activity of inhibitors of beta-1,3-glucan synthesis. Antimicrob Agents Chemother. 2012; 56:3428–3431. [PubMed: 22391552]

Coffman JA, Rai R, Loprete DM, Cunningham T, Svetlov V, Cooper TG. Cross regulation of four GATA factors that control nitrogen catabolic gene expression in *Saccharomyces cerevisiae*. J Bacteriol. 1997; 179:3416–3429. [PubMed: 9171383]

Cox GM, Mukherjee J, Cole GT, Casadevall A, Perfect JR. Urease as a virulence factor in experimental cryptococcosis. Infect Immun. 2000; 68:443–448. [PubMed: 10639402]

Crespo JL, Powers T, Fowler B, Hall MN. The TOR-controlled transcription activators GLN3, RTG1, and RTG3 are regulated in response to intracellular levels of glutamine. Proc Natl Acad Scie USA. 2002; 99:6784–6789.

Cruz MC, Cavallo LM, Görlach JM, Cox G, Perfect JR, Cardenas ME, et al. Rapamycin antifungal action is mediated via conserved complexes with FKBP12 and TOR kinase homologs in *Cryptococcus neoformans*. Mol Cell Biol. 1999; 19:4101–4112. [PubMed: 10330150]

Cruz Martho KF, de Melo AT, Takahashi JP, Guerra JM, Santos DC, Purisco SU, et al. Amino acid permeases and virulence in *Cryptococcus neoformans*. PLoS One. 2016; 11:e0163919. [PubMed: 27695080]

Dabas N, Schneider S, Morschhäuser J. Mutational analysis of the *Candida albicans* ammonium permease Mep2p reveals residues required for ammonium transport and signalling. Eukaryot Cell. 2009; 8:147–160. [PubMed: 19060183]

Denning DW, Bromley MJ. Infectious Disease. How to bolster the antifungal pipeline. Science. 2015; 27:1414–1416.

De Souza WR, Morais ER, Krohn NG, Savoldi M, Goldman MH, Rodrigues F, et al. Identification of metabolic pathways influences by the G-protein coupled receptors GprB and GprD in *Aspergillus nidulans*. PLoS One. 2013; 8:e62088. [PubMed: 23658706]

De Virgilio C, Loewith R. The TOR signalling network from yeast to man. Int J Biochem Cell Biol. 2006; 38:1476–1481. [PubMed: 16647875]

De Vit MJ, Waddle JA, Johnston M. Regulated nuclear translocation of the Mig1 glucose repressor. Mol Biol Cell. 1997; 8:1603–1618. [PubMed: 9285828]

Donaton MC, Holsbeeks I, Lagatie O, Van Zeebroeck G, Crauwels M, Winderickx J, Thevelein JM. The Gap1 general amino acid permease acts as an amino acid sensor for activation of protein kinase A targets in the yeast *Saccharomyces cerevisiae*. Mol Microbiol. 2003; 50:911–929. [PubMed: 14617151]

Dowzer CE, Kelly JM. Analysis of the *creA* gene, a regulator of carbon catabolite repression in *Aspergillus nidulans*. Mol Cell Biol. 1991; 11:5701–5709. [PubMed: 1922072]

Ene IV, Adya AK, Wehmeier S, Brand AC, MacCallum DM, Gow NA, et al. Host carbon sources modulate cell wall architecture, drug resistance and virulence in a fungal pathogen. Cell Microbiol. 2012; 14:1319–1335. [PubMed: 22587014]

Ene IV, Cheng SC, Netea MG, Brown AJ. Growth of *Candida albicans* cells on the physiologically relevant carbon source lactate affects their recognition and phagocytosis by immune cells. Infect Immun. 2013; 81:238–248. [PubMed: 23115042]

Fan J, Chaturvedi V, Shen SH. Identification and phylogenetic analysis of a glucose transporter gene family from the human pathogenic yeast *Candida albicans*. J Mol Evol. 2002; 55:336–346. [PubMed: 12187386]

Fanning S, Xu W, Solis N, Woolford CA, Filler SG, Mitchell AP. Divergent targets of *Candida albicans* biofilm regulator Bcr1 *in vitro* and *in vivo*. Eukaryot Cell. 2012; 11:896–904. [PubMed: 22544909]

Farnell E, Rousseau K, Thornton DJ, Bowyer P, Herrick SE. Expression and secretion of *Aspergillus fumigatus* proteases are regulated in response to different protein substrates. Fungal Biol. 2012; 116:1003–1012. [PubMed: 22954343]

Fernandes JD, Martho K, Tofik V, Vallim MA, Pascon RC. The role of amino acid permeases and tryptophan biosynthesis in *Cryptococcus neoformans* survival. PLoS One. 2015; 10:e0132369. [PubMed: 26162077]

Fleck CB, Brock M. *Aspergillus fumigatus* catalytic glucokinase and hexokinase: expression analysis and importance for germination, growth, and conidiation. Eukaryot Cell. 2010; 9:1120–1135. [PubMed: 20453072]

Forsberg H, Ljungdahl PO. Sensors of extracellular nutrients in *Saccharomyces cerevisiae*. Curr Genet. 2001; 40:91–109. [PubMed: 11680826]

Fradin C, De Groot P, MacCallum D, Schaller M, Klis F, Odds FC, et al. Granulocytes govern the transcriptional response, morphology and proliferation of *Candida albicans* in human blood. Mol Microbiol. 2005; 56:397–415. [PubMed: 15813733]

Gancedo JM. Yeast carbon catabolite repression. Microbiol Mol Biol Rev. 1998; 62:334–361. [PubMed: 9618445]

García I, Gonzalez R, Gómez D, Scazzocchio C. Chromatin rearrangements in the *prnD-prnB* bidirectional promoter: dependence on transcription factors. Eukaryot Cell. 2004; 3:144–156. [PubMed: 14871945]

García I, Mathieu M, Nikolaev I, Felenbok B, Scazzocchio C. Roles of the *Aspergillus nidulans* homologues of Tup1 and Ssn6 in chromatin structure and cell viability. FEMS Microbiol Lett. 2008; 289:146–154. [PubMed: 19054105]

Gaston B, Ratjen F, Vaughan JW, Malhotra NR, Canady RG, Snyder AH, et al. Nitrogen redox balance in the cystic fibrosis airway: effects of antipseudomonal therapy. Am J Respir Crit Care Med. 2002; 165:387–390. [PubMed: 11818326]

Gautam P, Madan T, Gade WN, Sarma PU. Immunoproteomic analysis of secretory proteins of *Aspergillus fumigatus* with specific IGE immunoreactivity. Indian J Clin Biochem. 2006; 21:12–19. [PubMed: 23105607]

Gehrke A, Heinekamp T, Jacobson ID, Brakhage AA. Heptahelical receptors GprC and GprD of *Aspergillus fumigatus* are essential regulators of colony growth, hyphal morphogenesis, and virulence. Appl Environ Microbiol. 2010; 76:3989–3998. [PubMed: 20418440]

Grahl N, Puttikamonkul S, Macdonald JM, Gamcsik MP, Ngo LY, Hohl TM, et al. *In vivo* hypoxia and a fungal alcohol dehydrogenase influence the pathogenesis of invasive pulmonary aspergillosis. PLoS Pathog. 2011; 7:e1002145. [PubMed: 21811407]

Grice CM, Bertuzzi M, Bignell EM. Receptor-mediated signalling in *Aspergillus fumigatus*. Front Microbiol. 2013; 4doi: 10.3389/fmicb.2013.00026

Gropp K, Schild L, Schindler S, Hube B, Zipfel PF, Skerka C. The yeast *Candida albicans* evades human complement attack by secretion of aspartic proteases. Mol Immunol. 2009; 47:465–475. [PubMed: 19880183]

Gsaller F, Hortschansky P, Beattie SR, Klammer V, Tuppatsch K, Lechner BE, et al. The Janus transcription factor HapX controls fungal adaptation to both iron starvation and iron excess. EMBO J. 2014; 33:2261–2276. [PubMed: 25092765]

Han KH, Seo JA, Yu JH. A putative G protein-coupled receptor negatively controls sexual development in *Aspergillus nidulans*. Mol Microbiol. 2004; 51:1333–1345. [PubMed: 14982628]

Han X, Qiu M, Wang B, Yin WB, Nie X, Qin Q, Ren S, et al. Functional analysis of the nitrogen metabolite repression regulator gene *nmrA* in *Aspergillus flavus*. Front Microbiol. 2016; 7:1794. [PubMed: 27933036]

Hensel M, Arst HN Jr, Aufauvre-Brown A, Holden DW. The role of the *Aspergillus fumigatus areA* gene in invasive pulmonary aspergillosis. Mol Gen Genet. 1998; 258:553–557. [PubMed: 9669338]

Herrero P, Martinez-Campa C, Moreno F. The hexokinase 2 protein participates in regulatory DNA-protein complexes necessary for glucose repression of the *SUC2* gene in *Saccharomyces cerevisiae*. FEBS Lett. 1998; 434:71–76. [PubMed: 9738454]

Hicks J, Lockington RA, Strauss J, Dieringer D, Kubicek CP, Kelly J, et al. RcoA has pleiotropic effects on *Aspergillus nidulans* cellular development. Mol Microbiol. 2001; 39:1482–1493. [PubMed: 11260466]

Homann OR, Dea J, Noble SM, Johnson AD. A phenotypic profile of the *Candida albicans* regulatory network. PLoS Genet. 2009; 5:e1000783. [PubMed: 20041210]

Hu G, Cheng PY, Sham A, Perfect JR, Kronstad JW. Metabolic adaptation in *Cryptococcus neoformans* during early murine pulmonary infection. Mol Microbiol. 2008a; 69:1456–1475. [PubMed: 18673460]

Hu JZ, Rommereim DN, Minard KR, Woodstock A, Harrer BJ, Wind RA, et al. Metabolomics in lung inflammation:a high-resolution (1)h NMR study of mice exposed to silica dust. Toxicol Mech Methods. 2008b; 18:385–398. [PubMed: 20020862]

Hube B, Sanglard D, Odds FC, Hess D, Monod M, Schäfer W, Brown AJ, Gow NA. Disruption of each of the secreted aspartyl proteinase genes *SAP1, SAP2*, and *SAP3* of *Candida albicans* attenuates virulence. Infect Immun. 1997; 65:3529–3538. [PubMed: 9284116]

Hudson DA, Sciascia QL, Sanders RJ, Norris GE, Edwards PJ, Sullivan PA, et al. Identification of the dialyzable serum inducer of germ-tube formation in *Candida albicans*. Microbiology. 2004; 150:3041–3049. [PubMed: 15347762]

Hynes MJ, Kelly JM. Pleiotropic mutants of *Aspergillus nidulans* altered in carbon metabolism. Mol Gen Genet. 1977; 150:193–204. [PubMed: 320455]

Jacobson ES. Pathogenic roles for fungal melanins. Clin Microbiol Rev. 2000; 13:708–717. [PubMed: 11023965]

Jonkers W, Rep M. Mutation of *CRE1* in *Fusarium oxysporum* reverts the pathogenicity defects of the *FRP1* deletion mutant. Mol Microbiol. 2009; 74:1100–1113. [PubMed: 19912543]

Jung WH, Saikia S, Hu G, Wang J, Fung CK, D'Souza C, et al. HapX positively and negatively regulates the transcriptional response to iron deprivation in *Cryptococcus neoformans*. PLoS Pathog. 2010; 6:e1001209. [PubMed: 21124817]

Kelly JM, Hynes MJ. Increased and decreased sensitivity to carbon catabolite repression of enzymes of acetate metabolism in mutants of *Aspergillus nidulans*. Mol Gen Genet. 1977; 156:87–92. [PubMed: 23491]

Kim JH, Roy A, Jouandot D, Cho KH. The glucose signaling network in yeast. Biochim Biophys Acta. 2013; 1830:5204–5210. [PubMed: 23911748]

Klein CJ, Olsson L, Nielsen J. Glucose control in *Saccharomyces cerevisiae*: the role of Mig1 in metabolic functions. Microbiology. 1998; 144(Pt 1):13–24. [PubMed: 9467897]

Kmetzsch L, Staats CC, Simon E, Fonseca FL, Oliveira DL, Joffe LS, et al. The GATA-type transcriptional activator Gat1 regulates nitrogen uptake and metabolism in the human pathogen *Cryptococcus neoformans*. Fungal Genet Biol. 2011; 48:192–199. [PubMed: 20673806]

Kogan TV, Jadoun J, Mittelman L, Hirschberg K, Osherov N. Involvement of secreted *Aspergillus fumigatus* proteases in disruption of the actin fiber cytoskeleton and loss of focal adhesion sites in infected A549 lung pneumocytes. J Infect Dis. 2004; 189:1965–1973. [PubMed: 15143461]

Kolattukudy PE, Lee JD, Rogers LM, Zimmerman P, Ceselski S, Fox B, et al. Evidence for possible involvement of an elastolytic serine protease in aspergillosis. Infect Immun. 1993; 61:2357–2368. [PubMed: 8500876]

Kraidlova L, Van Zeebroeck G, Van Dijck P, Sychrová H. The *Candida albicans* GAP gene family encodes permeases involved in general and specific amino acid uptake and sensing. Eukaryot Cell. 2011; 10:1219–1229. [PubMed: 21764911]

Kronstad JW, Attarian R, Cadieux B, Choi J, D'Souza CA, Griffiths EJ, et al. Expanding fungal pathogenesis: *Cryptococcus* breaks out of the opportunistic box. Nat Rev Microbiol. 2011; 9:193–203. [PubMed: 21326274]

Kronstad J, Saikia S, Nielson ED, Kretschmer M, Jung W, Hu G, et al. Adaptation of *Cryptococcus neoformans* to mammalian hosts: integrated regulation of metabolism and virulence. Eukaryot Cell. 2012; 11:109–118. [PubMed: 22140231]

Kulmburg P, Mathieu M, Dowzer C, Kelly J, Felenbok B. Specific binding sites in the *alcR* and *alcA* promoters of the ethanol regulon for the CREA repressor mediating carbon catabolite repression in *Aspergillus nidulans*. Mol Microbiol. 1993; 7:847–857. [PubMed: 8483416]

Jiménez-López C, Lorenz MC. Fungal immune evasion in a model host-pathogen interaction: *Candida albicans* versus macrophages. PLoS Pathog. 2013; 11:e1003741.

Jung MG, Kim SS, Yu JH, Shin KS. Characterization of *gprK* encoding a putative hybrid G-protein-coupled receptor in *Aspergillus fumigatus*. PLoS One. 2016; 11:e0161312. [PubMed: 27584150]

Langdon T, Sheerins A, Ravagnani A, Gielkens M, Caddick MX, Arst HN Jr. Mutational analysis reveals dispensability of the N-terminal region of the *Aspergillus* transcription factor mediating nitrogen metabolite repression. Mol Microbiol. 1995; 17:877–888. [PubMed: 8596437]

Lee H, Chang YC, Kwon-Chung KJ. *TUP1* disruption reveals biological differences between MATa and MATalpha strains of *Cryptococcus neoformans*. Mol Microbiol. 2005; 55:1222–1232. [PubMed: 15686566]

Lee H, Chang YC, Nardone G, Kwon-Chung KJ. *TUP1* disruption in *Cryptococcus neoformans* uncovers a peptide-mediated density-dependent growth phenomenon that mimics quorum sensing. Mol Microbiol. 2007; 64:591–601. [PubMed: 17462010]

Lee H, Chang YC, Varma A, Kwon-Chung KJ. Regulatory diversity of *TUP1* in *Cryptococcus neoformans*. Eukaryot Cell. 2009; 8:1901–1908. [PubMed: 19820119]

Lee H, Khanal Lamichhane A, Garraffo HM, Kwon-Chung KJ, Chang YC. Involvement of PDK1, PKC and TOR signalling pathways in basal fluconazole tolerance in *Cryptococcus neoformans*. Mol Microbiol. 2012; 84:130–146. [PubMed: 22339665]

Lee IR, Lui EY, Chow EW, Arras SD, Morrow CA, Fraser JA. Reactive oxygen species homeostasis and virulence of the fungal pathogen *Cryptococcus neoformans* requires an intact proline catabolism pathway. Genetics. 2013; 194:421–433. [PubMed: 23564202]

Lee IR, Morrow CA, Fraser JA. Nitrogen regulation of virulence in clinically prevalent fungal pathogens. FEMS Microbiol Lett. 2013; 345:77–84. [PubMed: 23701678]

Lee CM, Nantel A, Jiang L, Whiteway M, Shen SH. The serine/threonine protein phosphatase SIT4 modulates yeast-to-hypha morphogenesis and virulence in *Candida albicans*. Mol Microbiol. 2004; 51:691–709. [PubMed: 14731272]

Lee IR, Yang L, Sebetso G, Allen R, Doan TH, Blundell R, et al. Characterization of the complete uric acid degradation pathway in the fungal pathogen *Cryptococcus neoformans*. PLoS One. 2013; 8:e64292. [PubMed: 23667704]

Li L, Shen G, Zhang ZG, Wang YL, Thompson JK, Wang P. Canonical heterotrimeric G proteins regulating mating and virulence in *Cryptococcus neoformans*. Mol Biol Cell. 2007; 18:4201–4209. [PubMed: 17699592]

Liao WL, Ramón AM, Fonzi WA. *GLN3* encodes a global regulator of nitrogen metabolism and virulence of *C. albicans*. Fungal Genet Biol. 2008; 45:514–526. [PubMed: 17950010]

Limjindaporn T, Khalaf RA, Fonzi WA. Nitrogen metabolism and virulence of *Candida albicans* require the GATA-type transcriptional activator encoded by *GAT1*. Mol Microbiol. 2003; 50:993–1004. [PubMed: 14617156]

Liu TB, Wang Y, Baker GM, Fahmy H, Jiang L, Xue C. The glucose sensor-like protein Hxs1 is a high-affinity glucose transporter and required for virulence in *Cryptococcus neoformans*. PLoS One. 2013; 8:e64239. [PubMed: 23691177]

Lockington RA, Kelly JM. The WD40-repeat protein CreC interacts with and stabilizes the deubiquitinating enzyme CreB *in vivo* in *Aspergillus nidulans*. Mol Microbiol. 2002; 43:1173–1182. [PubMed: 11918805]

Lopez-Berges MS, Capilla J, Turra D, Schafferer L, Matthijs S, Jochl C, et al. HapX-mediated iron homeostasis is essential for rhizosphere competence and virulence of the soilborne pathogen *Fusarium oxysporum*. Plant Cell. 2012; 24:3805–3822. [PubMed: 22968717]

Lorenz MC, Bender JA, Fink GR. Transcriptional response of *Candida albicans* upon internalization by macrophages. Eukaryot Cell. 2004; 3:1076–1087. [PubMed: 15470236]

Machida M, Asai K, Sano M, Tanaka T, Kumagai T, Terai G, et al. Genome sequencing and analysis of *Aspergillus oryzae*. Nature. 2005; 438:1157–1161. [PubMed: 16372010]

Magasanik B, Kaiser CA. Nitrogen regulation in *Saccharomyces cerevisiae*. Gene. 2002; 290:1–18. [PubMed: 12062797]

Maidan MM, De Rop L, Serneels J, Exler S, Rupp S, Tournu H, et al. The G protein-coupled receptor Gpr1 and the Galpha protein Gpa2 act through the cAMP-protein kinase A pathway to induce morphogenesis in *Candida albicans*. Mol Biol Cell. 2005a; 16:1971–1986. [PubMed: 15673611]

Maidan MM, Thevelein JM, Van Dijck P. Carbon source induced yeast-to-hypha transition in *Candida albicans* is dependent on the presence of amino acids and on the G-protein-coupled receptor Gpr1. Biochem Soc Trans. 2005b; 33:291–300. [PubMed: 15667329]

Margelis S, D'Souza C, Small AJ, Hynes MJ, Adams TH, Davis MA. Role of glutamine synthetase in nitrogen metabolite repression in *Aspergillus nidulans*. J Bacteriol. 2001; 183:5826–5833. [PubMed: 11566979]

Mayer FL, Wilson D, Hube B. *Candida albicans* pathogenicity mechanisms. Virulence. 2013; 4:119–128. [PubMed: 23302789]

McClelland EE, Bernhardt P, Casadevall A. Coping with multiple virulence factors: which is most important? PLoS Pathog. 2005; 1:e40. [PubMed: 16738705]

McDonagh A, Fedorova ND, Crabtree J, Yu Y, Kim S, Chen D, et al. Sub-telomere directed gene expression during initiation of invasive aspergillosis. PLoS Pathog. 2008; 4:e1000154. [PubMed: 18787699]

Meyer V, Andersen MR, Brakhage AA, Braus GH, Caddick MX, Cairns TC, et al. Current challenges of research on filamentous fungi in relation to human welfare and a sustainable bio-economy: a white paper. Fungal Biol Biotechnol. 2016; 3doi: 10.1186/s40694-016-0024-8

Miwa T, Takagi Y, Shinozaki M, Yun CW, Schell WA, Perfect JR, et al. Gpr1, a putative G-protein-coupled receptor, regulates morphogenesis and hypha formation in the pathogenic fungus *Candida albicans*. Eukaryot Cell. 2004; 3:919–931. [PubMed: 15302825]

Mizuno T, Masuda Y, Irie K. The *Saccharomyces cerevisiae* AMPK, Snf1, negatively regulates the Hog1 MAPK pathway in ER stress response. PLoS Genet. 2015; 11:e1005491. [PubMed: 26394309]

Mogensen J, Nielsen HB, Hofmann G, Nielsen J. Transcription analysis using high-density micro-arrays of *Aspergillus nidulans* wild-type and *creA* mutant during growth on glucose or ethanol. Fungal Genet Biol. 2005; 43:593–603.

Monod M, Capoccia S, Léchenne B, Zaugg C, Holdom M, Jousson O. Secreted proteases from pathogenic fungi. Int J Med Microbiol. 2002; 292:405–419. [PubMed: 12452286]

Morton CO, Varga JJ, Hornbach A, Mezger M, Sennefelder H, Kneitz S, et al. The temporal dynamics of differential gene expression in *Aspergillus fumigatus* interacting with human immature dendritic cells in vitro. PLoS One. 2011; 6:e16016. [PubMed: 21264256]

Murad AM, d'Enfert C, Gaillardin C, Tournu H, Tekaia F, Talibi D, et al. Transcript profiling in *Candida albicans* reveals new cellular functions for the transcriptional repressors CaTup1, CaMig1 and CaNrg1. Mol Microbiol. 2001; 42:981–993. [PubMed: 11737641]

Murad AM, Leng P, Straffon M, Wishart J, Macaskill S, MacCallum D, et al. *NRG1* represses yeast-hypha morphogenesis and hypha-specific gene expression in *Candida albicans*. EMBO J. 2001; 20:4742–4752. [PubMed: 11532938]

Naglik JR, Albrecht A, Bader O, Hube B. *Candida albicans* proteinases and host/pathogen interactions. Cell Microbiol. 2004; 6:915–926. [PubMed: 15339267]

Naglik JR, Challacombe SJ, Hube B. *Candida albicans* secreted aspartyl proteinases in virulence and pathogenesis. Microbiol Mol Biol Rev. 2003; 67:400–428. [PubMed: 12966142]

Naglik JR, Moyes D, Makwana J, Kanzaria P, Tsichlaki E, Weindl G, et al. Quantitative expression of the *Candida albicans* secreted aspartyl proteinase gene family in human oral and vaginal candidiasis. Microbiology. 2008; 154:3266–3280. [PubMed: 18957581]

Nett JE, Lepak AJ, Marchillo K, Andes DR. Tim course global expression analysis of an *in vivo Candida* biofilm. J Infect Dis. 2009; 200:307–313. [PubMed: 19527170]

Nierman WC, Pain A, Anderson MJ, Wortman JR, Kim HS, Arroyo J, et al. Genome sequence of the pathogenic and allergenic filamentous fungus *Aspergillus fumigatus*. Nature. 2005; 438:1151–1156. [PubMed: 16372009]

Olszewski MA, Noverr MC, Chen GH, Toews GB, Cox GM, Perfect JR, et al. Urease expression by *Cryptococcus neoformans* promotes microvascular sequestration, thereby enhancing central nervous system invasion. Am J Pathol. 2004; 164:1761–1771. [PubMed: 15111322]

Oosthuizen JL, Gomez P, Ruan J, Hackett TL, Moore MM, Knight DA, et al. Dual organism transcriptomics of airway epithelial cells interacting with conidia of *Aspergillus fumigatus*. PLoS One. 2011; 6:e20527. [PubMed: 21655222]

Ostling J, Ronne H. Negative control of the Mig1p repressor by Snf1p-dependent phosphorylation in the absence of glucose. Eur J Biochem. 1998; 252:162–168. [PubMed: 9523726]

Ozcan S, Dover J, Johnston M. Glucose sensing and signaling by two glucose receptors in the yeast *Saccharomyces cerevisiae*. EMBO J. 1998; 17:2566–2573. [PubMed: 9564039]

Panepinto JC, Oliver BG, Fortwendel JR, Smith DL, Askew DS, Rhodes JC. Deletion of the *Aspergillus fumigatus* gene encoding the Ras-related protein RhbA reduces virulence in a model of Invasive pulmonary aspergillosis. Infect Immun. 2003; 71:2819–2826. [PubMed: 12704156]

Peeters, K., Thevelein, JM. Glucose Sensing and Signal Transduction in *Saccharomyces cerevisiae*. In: Piškur, J., Compagno, C., editors. Molecular Mechanisms in Yeast Carbon Metabolism. Berlin, Heidelberg: Springer Berlin Heidelberg; 2014. p. 21-56.

Petter R, Chang YC, Kwon-Chung KJ. A gene homologous to *Saccharomyces cerevisiae* SNF1 appears to be essential for the viability of *Candida albicans*. Infect Immun. 1997; 65:4909–4917. [PubMed: 9393775]

Philips BJ, Meguer JX, Redman J, Baker EH. Factors determining the appearance of glucose in upper and lower respiratory tract secretions. Intensive Care Med. 2003; 29:2204–2210. [PubMed: 14647890]

Portnoy T, Margeot A, Linke R, Atanasova L, Fekete E, Sándor E, et al. The CRE1 carbon catabolite repressor of the fungus *Trichoderma reesei*: a master regulator of carbon assimilation. BMC Genomics. 2011; 12:269. [PubMed: 21619626]

Price MS, Betancourt-Quiroz M, Price JL, Toffaletti DL, Vora H, Hu G, et al. *Cryptococcus neoformans* requires a functional glycolytic pathway for disease but not persistence in the host. MBio. 2011; 2:e00103–00111. [PubMed: 21652778]

Puri S, Kumar R, Chadha S, Tati S, Conti HR, Hube B, et al. Secreted aspartic protease cleavage of *Candida albicans* Msb2 activates Cek1 MAPK signaling affecting biofilm formation and oropharyngeal candidiasis. PLoS One. 2012; 7:e46020. [PubMed: 23139737]

Ramachandra S, Linde J, Brock M, Guthke R, Hube B, Brunke S. Regulatory networks controlling nitrogen sensing and uptake in *Candida albicans*. PLoS One. 2014; 9:e92734. [PubMed: 24651113]

Richardson AJ, McKain N, Wallace RJ. Ammonia production by human faecal bacteria, and the enumeration, isolation and characterization of bacteria capable of growth on peptides and amino acids. BMC Microbiol. 2013; 13:6. [PubMed: 23312016]

Ries LN, Beattie SR, Espeso EA, Cramer RA, Goldman GH. Diverse Regulation of the CreA Carbon Catabolite Repressor in Aspergillus nidulans. Genetics. 2016; 203:335–352. [PubMed: 27017621]

Rohde JR, Bastidas R, Puria R, Cardenas ME. Nutritional control via Tor signaling in *Saccharomyces cerevisiae*. Curr Opin Microbiol. 2008; 11:153–160. [PubMed: 18396450]

Ruijter GJ, Visser J. Carbon repression in Aspergilli. FEMS Microbiol Lett. 1997; 151:103–114. [PubMed: 9228741]

Rutherford JC, Lin X, Nielsen K, Heitman J. Amt2 permease is required to induce ammonium-responsive invasive growth and mating *in Cryptococcus neoformans*. Eukaryot Cell. 2008; 7:237–246. [PubMed: 18055915]

Sandai D, Zhikang Y, Selway L, Stead D, Walker J, Leach MD, et al. The evolutionary rewiring of ubiquitination targets has reprogrammed the regulation of carbon assimilation in the pathogenic yeast *Candida albicans*. MBio. 2012; 3:e00495–12. [PubMed: 23232717]

Sanglard D, Hube B, Monod M, Odds FC, Gow NA. A triple deletion of the secreted aspartyl proteinase genes *SAP4, SAP5*, and *SAP6* of *Candida albicans* causes attenuated virulence. Infect Immun. 1997; 65:3539–3546. [PubMed: 9284117]

Schrettl M, Beckmann N, Varga J, Heinekamp T, Jacobsen ID, Jochl C, et al. HapX-mediated adaption to iron starvation is crucial for virulence of *Aspergillus fumigatus*. PLoS Pathog. 2010; 6:e1001124. [PubMed: 20941352]

Schug ZT, Vande Voorde J, Gottlieb E. The metabolic fate of acetate in cancer. Nat Rev Cancer. 2016; 11:708–717.

Schure EG, van Riel NA, Verrips CT. The role of ammonia metabolism in nitrogen catabolite repression in *Saccharomyces cerevisiae*. FEMS Microbiol Rev. 2000; 24:67–83. [PubMed: 10640599]

Seehusen DA, Reeves MM, Fomin DA. Cerebrospinal fluid analysis. Am Fam Physician. 2003; 68:1103–1108. [PubMed: 14524396]

Sexton JA, Brown V, Johnston M. Regulation of sugar transport and metabolism by the *Candida albicans* Rgt1 transcriptional repressor. Yeast. 2007; 24:847–860. [PubMed: 17605131]

Sharon H, Hagag S, Osherov N. Transcription factor PrtT controls expression of multiple secreted proteases in the human pathogenic mold *Aspergillus fumigatus*. Infect Immun. 2009; 77:4051–4060. [PubMed: 19564385]

Shi M, Li SS, Zheng C, Jones GJ, Kim KS, Zhou H, et al. Real-time imaging of trapping and urease-dependent transmigration of *Cryptococcus neoformans* in mouse brain. J Clin Invest. 2010; 120:1683–1693. [PubMed: 20424328]

Staschke KA, Dey S, Zaborske JM, Palam LR, McClintick JN, Pan T, et al. Integration of general amino acid control and target of rapamycin (TOR) regulatory pathways in nitrogen assimilation in yeast. J Biol Chem. 2010; 285:16893–16911. [PubMed: 20233714]

Steen BR, Zuyderduyn S, Toffaletti DL, Marra M, Jones SJ, Perfect JR, et al. *Cryptococcus neoformans* gene expression during experimental cryptococcal meningitis. Eukaryot Cell. 2003; 2:1336–1349. [PubMed: 14665467]

Stumvoll M, Perriello G, Meyer C, Gerich J. Role of glutamine in human carbohydrate metabolism in kidney and other tissues. Kidney Int. 1999; 55:778–792. [PubMed: 10027916]

Su C, Lu Y, Liu H. Reduced TOR signaling sustains hyphal development in *Candida albicans* by lowering Hog1 basal activity. Mol Biol Cell. 2013; 24:385–397. [PubMed: 23171549]

Sugui JA, Kim HS, Zarember KA, Chang YC, Gallin JI, Nierman WC, et al. Genes differentially expressed in conidia and hyphae of *Aspergillus fumigatus* upon exposure to human neutrophils. PLoS One. 2008; 3:e2655. [PubMed: 18648542]

Sun J, Glass NL. Identification of the CRE-1 cellulolytic regulon in *Neurospora crassa*. PLoS One. 2011; 6:e25654. [PubMed: 21980519]

Svetlov VV, Coppe TG. The *Saccharomyces cerevisiae* GATA factors Dal80p an Deh1p can form homo- and heterodimeric complexes. J Bacteriol. 1998; 180:5682–5688. [PubMed: 9791119]

Tamayo EN, Villanueva A, Hasper AA, de Graaff LH, Ramón D, Orejas M. CreA mediates repression of the regulatory gene *xlnR* which controls the production of xylanolytic enzymes in *Aspergillus nidulans*. Fungal Genet Biol. 2008; 45:984–993. [PubMed: 18420433]

Tanaka M, Hiramoto T, Tada H, Shintani T, Gomi K. Improved α-amylase production by dephosphorylation mutation of CreD, an arrestin-like protein required for glucose-induced endocytosis of maltose permease and carbon catabolite derepression in *Aspergillus oryzae*. Appl Environ Microbiol. 2017; 83:e00592–17. [PubMed: 28455339]

Todd RB, Lockington RA, Kelly JM. The *Aspergillus nidulans creC* gene involved in carbon catabolite repression encodes a WD40 repeat protein. Mol Gen Genet. 2000; 263:561–570. [PubMed: 10852476]

Tsao CC, Chen YT, Lan CY. A small G protein Rhb1 and a GTPase-activating protein Tsc2 involved in nitrogen starvation-induced morphogenesis and cell wall integrity of *Candida albicans*. Fungal Genet Biol. 2009; 46:126–136. [PubMed: 19095072]

Tudzynski B. Nitrogen regulation of fungal secondary metabolism in fungi. Front Microbiol. 2014; 28doi: 10.3389/fmicb.2014.00656

Vu K, Tham R, Uhrig JP, Thompson GR 3rd, Na Pombeira S, Jamklang M, et al. Invasion of the central nervous system by *Cryptococcus neoformans* requires a secreted fungal metalloprotease. MBio. 2014; 5:e01101–e01114. [PubMed: 24895304]

Vylkova S, Lorenz MC. Modulation of phagosomal pH by *Candida albicans* promotes hyphal morphogenesis and requires Stp2p, a regulator of amino acid transport. PLoS Pathog. 2014; 10:e1003995. [PubMed: 24626429]

Wei H, Vienken K, Weber R, Bunting S, Requena N, Fischer R. A putative high affinity hexose transporter, *hxtA* of *Aspergillus nidulans* is induced in vegetative hyphae upon starvation and in ascogenous hyphae during cleistothecium formation. Fungal Genet Biol. 2004; 41:148–156. [PubMed: 14732261]

Wenjie X, Solis NV, Ehrlich RL, Woolford CA, Filler SG, Mitchell AP. Activation and alliance of regulatory pathways in *C. albicans* during mammalian infection. PLoS Biol. 2015; 13:e1002076. [PubMed: 25693184]

Wong KH, Hynes MJ, Davis MA. Recent advances in nitrogen regulation: a comparison between Saccharomyces cerevisiae and filamentous fungi. Eukaryot Cell. 2008; 7:917–925. [PubMed: 18441120]

Wong KH, Hynes MJ, Todd RB, Davis MA. Transcriptional control of *nmrA* by the bZIP transcription factor MeaB reveals a new level of nitrogen regulation in *Aspergillus nidulans*. Mol Microbiol. 2007; 66:534–551. [PubMed: 17854403]

Wong KH, Hynes MJ, Todd RB, Davis MA. Deletion and overexpression of the *Aspergillus nidulans* GATA factor AreB reveals unexpected pleiotropy. Microbiology. 2009; 155:3868–3880. [PubMed: 19628561]

Xu CY, Zhu HM, Wu JH, Wen H, Liu CJ. Increased permeability of blood-brain barrier is mediated by serine protease during *Cryptococcus* meningitis. J Int Med Res. 2014; 42:85–92. [PubMed: 24398759]

Xue C, Bahn YS, Cox GM, Heitman J. G protein-coupled receptor Gpr4 senses amino acids and activates the cAMP-PKA pathway in *Cryptococcus neoformans*. Mol Biol Cell. 2006; 17:667–679. [PubMed: 16291861]

Yang J, Li D, Liu X, Pan J, Yan B, Zhu X. Regulation of virulence factors, carbon utilization and virulence by SNF1 in *Cryptococcus neoformans* JEC21 and divergent actions of SNF1 between cryptococcal strains. Fungal Genet Biol. 2010; 47:994–1000. [PubMed: 20719250]

Zaragoza O, Rodriguez C, Gancedo C. Isolation of the MIG1 gene from *Candida albicans* and effects of its disruption on catabolite repression. J Bacteriol. 2000; 182:320–326. [PubMed: 10629176]

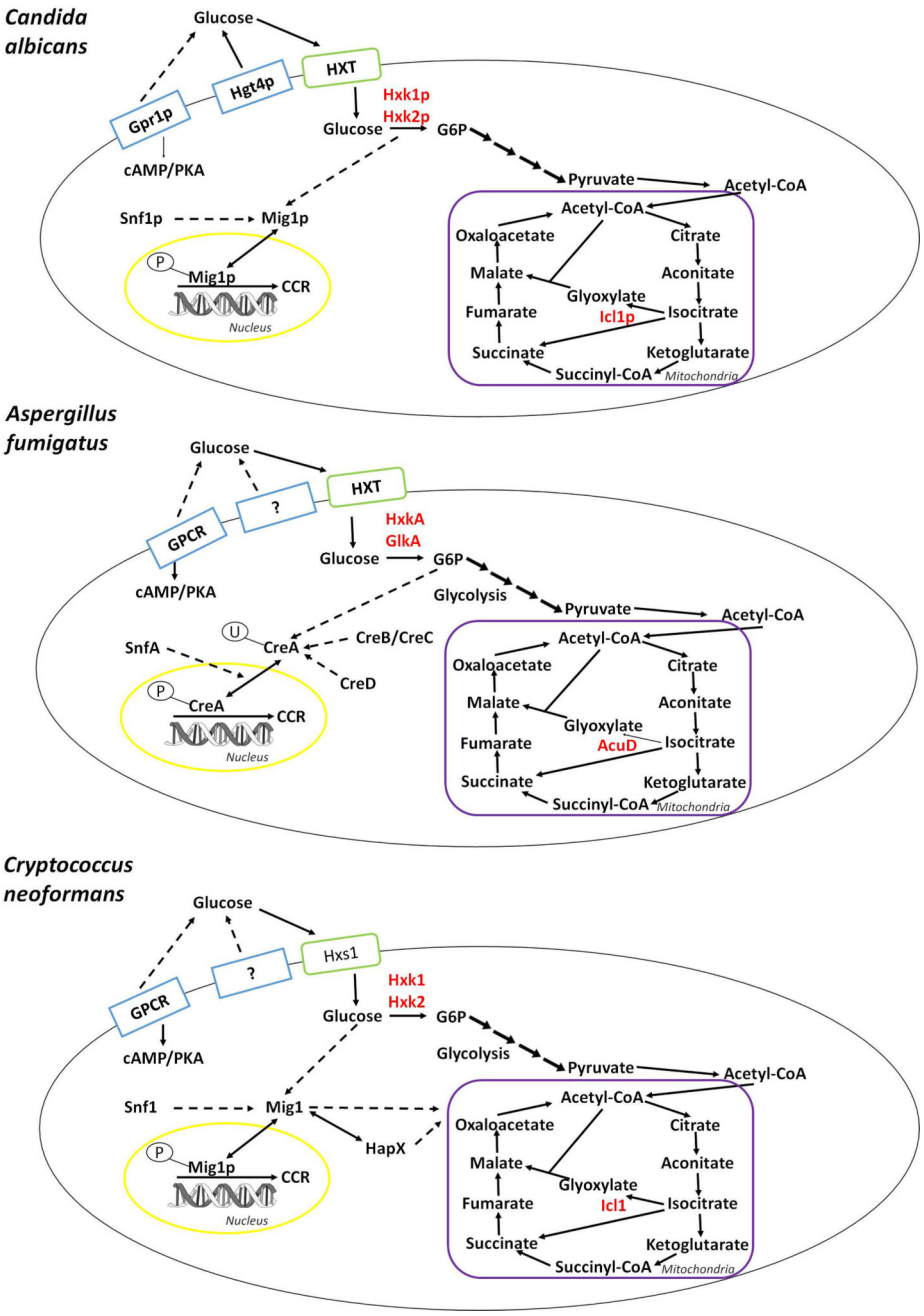

Figure 1.

Glucose sensing, uptake and metabolic pathways in *C. albicans, A. fumigatus* and *C. neoformans* (blue boxes: confirmed and putative sensors; green boxes: confirmed and putative transporters; red = metabolic enzymes, solid arrows = confirmed cellular processes, dashed arrows = cellular processes that are not elucidated; yellow = nucleus; purple = mitochondria). Glucose is sensed and taken up into the cell by specific hexose transporters (HXT) and is subsequently phosphorylated by the carbohydrate kinases hexokinase (HXK) or glucokinase (GLK). Further metabolic utilisation occurs via glycolysis and the TCA (tricarboxylic acid) cycle. Glucose uptake and subsequent phosphorylation serve as signals

for the carbon catabolite repressor (CCR) Mig1p/CreA/Mig1 to translocate to the nucleus and repress target genes. Several factors, such as the protein kinase SNF, the de-ubiquitinylation complex CreB/CreC or the transcriptional regulator of iron metabolism HapX, have been shown to be involved in CCR by either interacting directly or indirectly, with Mig1p/CreA/Mig1 in a species-dependent manner.

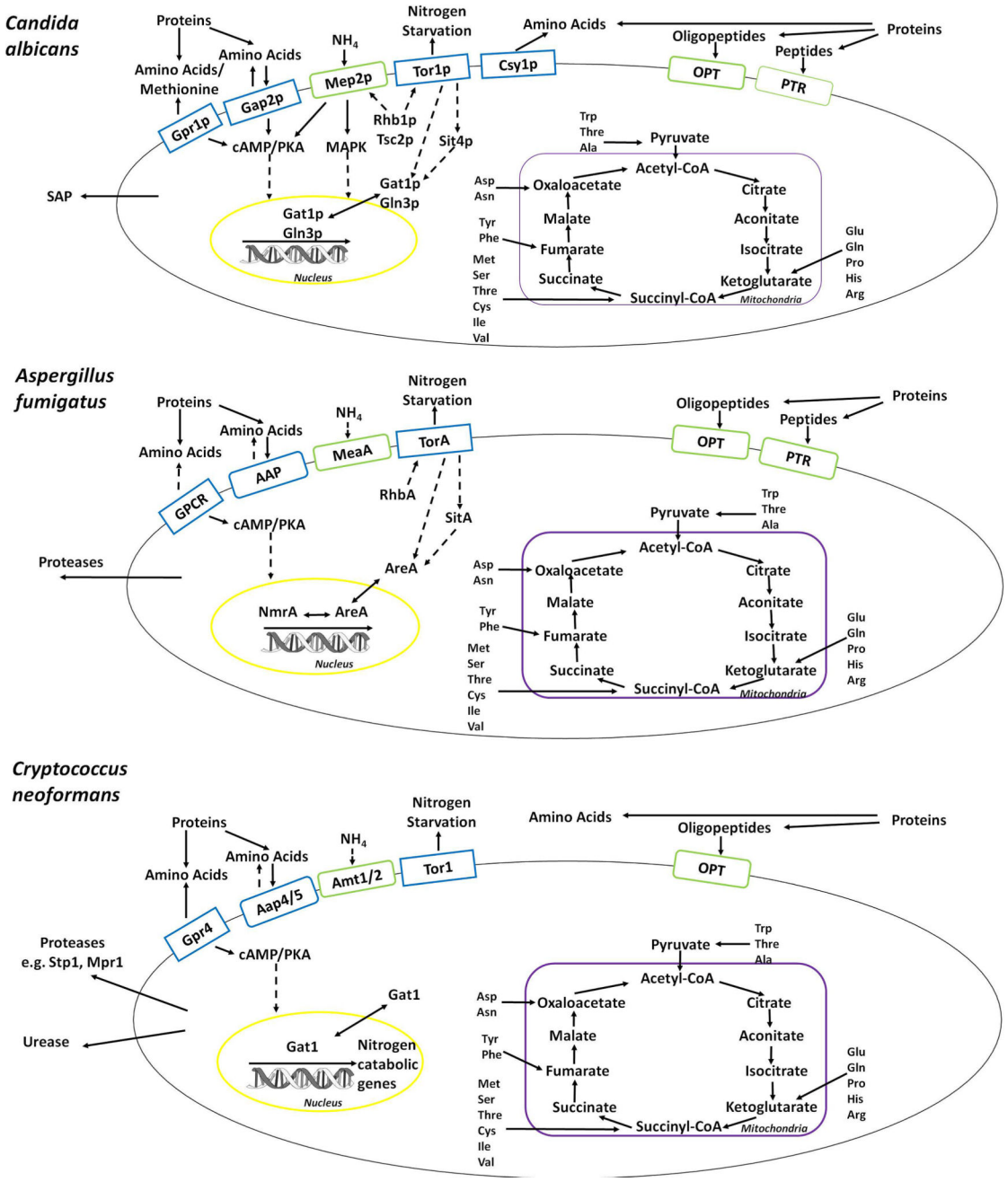

Figure 2.

Amino acid and ammonium sensing, uptake and metabolism in *C. albicans, A. fumigatus* and *C. neoformans* (blue boxes: confirmed and putative sensors; green boxes: confirmed and putative transporters; solid arrows = confirmed cellular processes, dashed arrows = cellular processes that are not elucidated; yellow = nucleus; purple = mitochondria). Proteins are degraded into peptides or proteins by secreted proteases (SAP = secreted aspartyl proteases). Peptides are internalised via oligopeptide (OPT) or peptide (PTR) transporters. Amino acids and ammonium are sensed and taken up by respective transporters and signals are relayed via several predicted pathways including cAMP and protein kinase A (PKA). Amino acids

serve as precursors for TCA (tricarboxylic acid) cycle intermediates to generate ATP. Transcription factors (Gat1p, Gln3p/AreA, NmrA/Gat1) are activated and translocate to the nucleus where they regulate the catabolic and anabolic utilisation of different nitrogen sources.

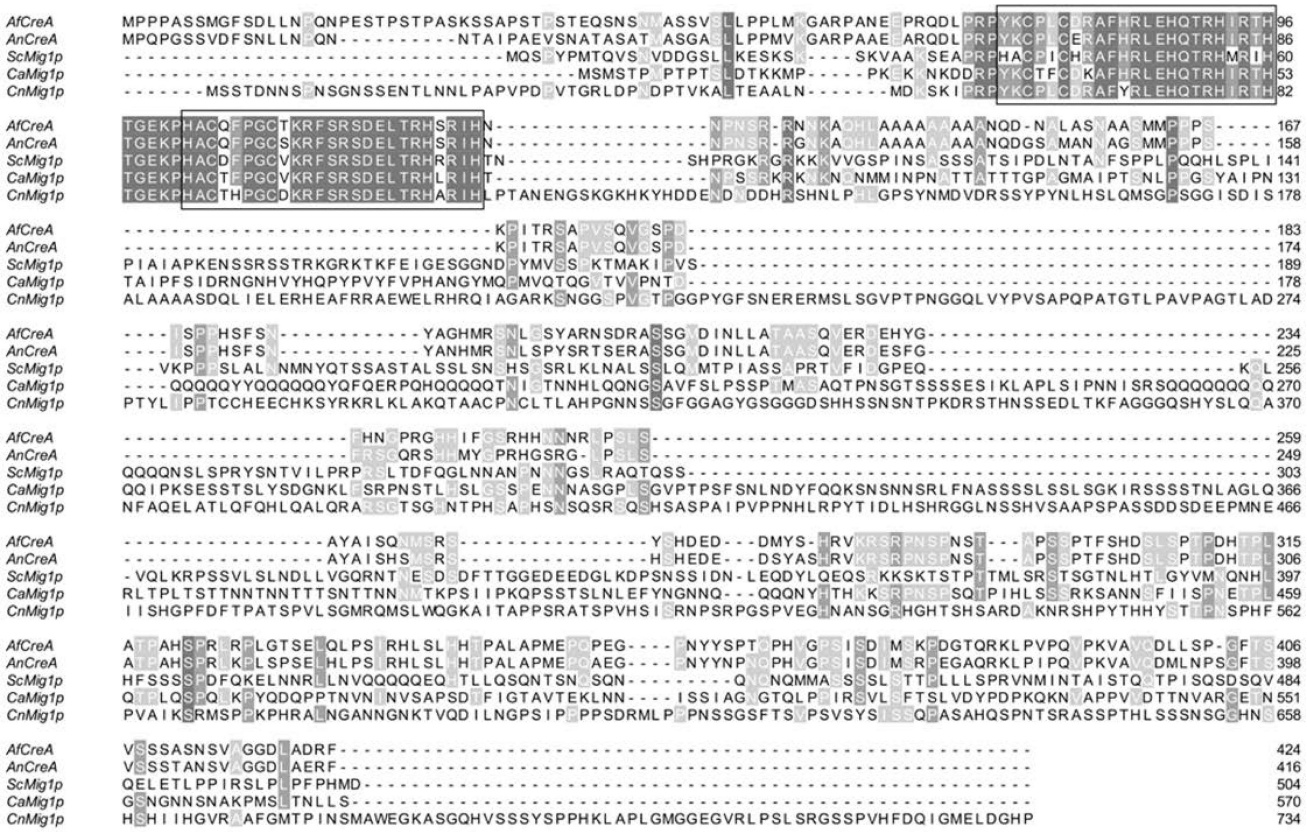

Figure 3.

ClustalW alignment of CreA/Mig1p/Mig1 amino acid sequences from *A. fumigatus* (AfCreA), *A. nidulans* (AnCreA), *S. cerevisiae* (ScMig1p), *C. albicans* (CaMig1p) and *C. neoformans* (CnMig1p). Percent identity is shown with shading, where darker shading indicates higher percent identity. Zinc-finger domains are outlined in black, based on SMART domain prediction of ScMig1p.

Mol Microbiol. Author manuscript; available in PMC 2019 February 01.

Table 1

Factors involved in glucose sensing and metabolism and their role in virulence, when compared to the respective wild-type (WT) strain, in the human pathogenic fungi *Aspergillus fumigatus*, *Cryptococcus neoformans* and *Candida albicans* (NC = not characterized).

S.c protein	Role/function	*A. fumigatus*		*C. neoformans*		*C. albicans*	
		Homolog	Virulence	Homolog	Virulence	Homolog	Virulence
Glucose sensing and uptake							
Gpr1p	GPCR	GprC	Delayed (Gehrke *et al.*, 2010)	Gpr1 (NC)	NC	Gpr1p	Context dependent (Miwa et al., 2004; Maidan *et al.*, 2005b)
Gpr1p	GPCR	GprD	Attenuated (Gehrke *et al.*, 2010)	-	-	-	-
-	GPCR	GprK	WT (wax moth larvae model; Jung *et al.*, 2016)	-	-	-	-
Gpa2p	G protein	NC	NC	Gpa2	WT (Li *et al.*, 2007)	Gpa2p	WT (Miwa *et al.*, 2004)
Snf3p	Glucose sensor	NC	NC	Hsx1	Delayed (Liu *et al.*, 2013)	Hgt4p	Delayed (Brown *et al.*, 2006)
Rgt2p	Glucose sensor	NC	NC	Hsx2 (essential)	NC		
Carbon catabolite repression							
Mig1p	Transcriptional repressor of CCR	CreA	Attenuated (Beattie *et al.*, 2017)	Mig1	WT (Caza *et al.*, 2016)	Mig1p	WT (Zaragoza *et al.*, 2000)
Tup1p	Co-repressor of CCR	Rco1	NC	Tup1	Delayed (Lee *et al.*, 2009)	Tup1p	Avirulent (Murad *et al.*, 2001)
Additional factors involved in CCR							
Snf1p	Protein kinase	SnfA	NC	SnfA	Avirulent/delayed (Hu *et al.*, 2008a; Yang *et al.*, 2010)	Snf1p (Essential)	NC/Heterozygous mutant - WT (Petter *et al.*, 1997)
Hxk2p	Hexokinase	HxkA/GlkA	NC	Hxk1/Hxk2	Avirulent -double mutant only (Price *et al.*, 2011)	Hxk2p	NC

S.c protein	Role/function	*A. fumigatus*		*C. neoformans*		*C. albicans*	
		Homolog	**Virulence**	**Homolog**	**Virulence**	**Homolog**	**Virulence**
-	De-ubiquitination enzyme	CreB	NC	-	-	Ubp13p	NC
-	WD-40 repeat protein	CreC	NC	-	-	Cop1p (C3_01720C_A)	NC
-	Arrestin	CreD	NC	-	-	Rod1p	NC

Mol Microbiol. Author manuscript; available in PMC 2019 February 01.

Table 2

Factors involved in ammonium and amino acid sensing and metabolism and their role in virulence, when compared to the respective wild-type (WT) strain, in the human pathogenic fungi *Aspergillus fumigatus*, *Cryptococcus neoformans* and *Candida albicans* (NC = not characterized).

		Homolog	Virulence	Homolog	Virulence	Homolog	Virulence
Nitrogen sensing and uptake							
Mep3p	Ammonium permease	MeaA	NC	Amt1	WT (Rutherford *et al.*, 2008)	Mep1p	NC
Mep2p	Ammonium permease	Afu1g10930	NC	Amt2	WT (Rutherford *et al.*, 2008)	Mep2p	NC
Ssy1p	Amino acid sensor	-	-	-	-	Csy1p	NC
Gap1p	Nitrogen sensor and amino acid permease	Afu7g04290	NC	Aap4 Aap5	Avirulent (Cruz Martho *et al.*, 2016)	Gap2p	NC
Tor1p, Tor2p	Protein kinase, major nitrogen sensor	TorA	NC	Tor1	NC	Tor1p	NC
Sit4p	Protein phosphatase	SitA	Avirulent (Bom *et al.*, 2015)	NC	NC	Sit4p	Attenuated (Lee *et al.*, 2004)
Rhb1p	GTPase	RhbA	Attenuated (Panepinto *et al.*, 2003)	NC	NC	Rhb1p	NC
Nitrogen catabolite repression (NCR)							
-	Co-repressor	NmrA	NC	-	-	-	-
Gat1p	GATA-type transcription factor	AreA	WT (Hensel *et al.*, 1998)	Gat1	WT (McClelland *et al.*, 2005)	Gat1p	Avirulent (Limjindaporn *et al.*, 2003)
Gln3p	GATA-type transcription factor	-	-	-	-	Gln3p	Attenuated (Liao *et al.*, 2008)
Gzf3p	GATA-type transcription factor	AreB	NC	-	-	Gzf3p	NC
-	Transcription factor	MeaB	NC	-	-	-	-
Additional factors involved in NCR							

Dur1p Dur2p	Urease	Afu1g04560 (UreB) Afu2g12900 (UreD)	NC	Ure1	Attenuated (Cox *et al.*, 2000)	Dur1p Dur2p	NC
Yps3p	Aspartic proteinase	OpsB	NC	-	-	Sap1p	Attenuated (Hube *et al.*, 1997)
Yps3p	Aspartic proteinase	OpsB	NC	-	-	Sap2p	Attenuated (Hube *et al.*, 1997)
Yps3p	Aspartic proteinase	OpsB	NC	-	-	Sap3p	Attenuated (Hube *et al.*, 1997)
Yps3p, Bar1p	Aspartic proteinases	OpsB	NC	-	-	Sap4p, Sap5p, Sap6p	Attenuated/Triple mutant only (Hube *et al.*, 1997)
-	Metalloproteinase	Mep/Aspf5	NC	Mpr1	Attenuated (Vu *et al.*, 2014)	-	-
Urc2p	Transcription factor	PrtT	WT (Bergmann *et al.*, 2009; Sharon *et al.*, 2009)	NC	NC	Zcf19p	NC